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ORIGINAL ARTICLES

THE EVOLUTION OF CLINICAL PATHOLOGY*

BY HARRY GAUSS, M.S., M.D., DENVER, COLO.

TO crystallize our theme we will arbitrarily define clinical pathology as that branch of medicine which seeks to correlate anatomic alterations with clinical symptoms by the refined methods of pathology, serology, bacteriology and chemistry. It follows almost as a corollary that the clinical pathologist functions within the practice of medicine in the diagnosis of disease rather than in the therapeusis of it. However, under certain extenuating circumstances the sphere of the clinical pathologist enlarges and he may supervise the management of certain conditions especially the infectious and contagious diseases where the diagnosis and control of the diseases is made largely by laboratory methods. Such a contingency arose during the World War when the Department of Laboratories and Infectious Diseases was organized in the American Expeditionary Force to handle the special situation.

Under ordinary existing conditions, however, the clinical pathologist operates in his laboratory where he combines the technical information of a multiplicity of sciences, or rather he appropriates such parts of the several sciences as he needs and combines them under the title of Clinical Pathology. While the several sciences he utilizes are regarded as distinct, if we are to believe the curriculums of the schools, we have only to trace them back a comparatively short time to find them originating in a common progenitor, the philosophy of the Middle Ages, and if traced a few centuries further back, the entire subject is lost in the religion and mysticism of the earlier races where its inception took place.

This attempt at the consolidation of miscellaneous portions of a group

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of sciences which originated from a common source is comparable to the efforts of some of our contemporaries in allied fields of endeavor. The modern physicist sees a merging of all sciences into a universal science. Physics, chemistry, biology and geology will cease to be independent sciences, lose their identities and adopt a common set of principles.

Modern science moves so rapidly that most of us are disturbed to find that the basic principles of the more exact sciences are not nearly so stable as it would be convenient to have them. The chemical element which only a short time ago, we learned, was the beginning of all inorganic and organic structures is no longer regarded as elemental; rather it is considered an intermediate state of organized energy. Even the molecule and atom into which they were split up are still further resolved into electrons and protons, two types of particles, both invisible but independently observable by certain effects they produce. Stars are born and die, much the same as organic life and the transmutation of metals is conceivable. The very elements are born and die, although their life cycle may be thousands or millions of years. There is nothing stable in the universe. Everything is in a state of transition or evolution, and every phenomenon must ultimately be explained in terms of a matter which is granular in structure and electrical in character (Mills). Emerson, the American essayist, must have contemplated a constantly changing universe when he wrote, "the gases gather to the solid firmament; the chemic lump arrives at the plant, and grows; arrives at the quadruped, and walks; arrives at the man, and thinks." So when we find the science of clinical pathology in an evolutionary phase we recognize that this is in accord with all natural law.

Clinical pathology may be said to have been born in May 22, 1922, at St. Louis, Mo., where the American Society of Clinical Pathologists was organized. It is true that clinical pathology existed before this time, but its consideration prior to this date must come under the heading of its embryology. However, since it is our purpose to trace its phylogeny as well as its ontogeny we may proceed under the title of the Evolution of Clinical Pathology.

Examination of the urine dates back to antiquity. We read in Garrison's "History of Medicine" that the Babylonians performed this procedure under certain conditions as a religious rite where it was combined with inspection of the viscera of sacrificed animals as an essential part of the augury conducted by the priest-physician. Terra cotta models of the liver have been found whose age is estimated at 3000 years, which indicated that this organ was used as a basis for forecasting the future. The Babylonians believed that the liver was the source of blood, hence the seat of the soul which was offered to the god and to inspect the liver was to approach divinity receiving human favors. The inscriptions on these models suggest that they regarded disease as being caused by demons. This belief was shared, as we know, by practically every early race. Moreover, the Babylonians mixed their notions of medicine with astrology, the two being intimately interrelated. The Babylonians probably followed the teaching of the earlier Persians accord-

ing to Meunier who states that Zoroaster, a mythical priestly lawgiver, taught that the liver was the site of human emotion. On the other hand, if the Babylonians were not keen physicians they were good soldiers; they conquered the Hebrews, whom they placed in captivity about 604 B.C. The rigid dietary laws adopted by the Jews which necessitated the slaughtering of cattle according to prescribed rule, also the rigorous examination of the viscera of these animals is recorded in their sacred writing, the Talmud, where is found record of the postmortems they conducted on slaughtered

Die Urinprobe by Gerard Dous, reproduced with the permission of the publishers from Hollander, Medizin in der klassichen Malerei, 3. Auflage, 1923, Verlag von Ferinand Enke in Stuttgart.

animals to determine their fitness for food. Diseased animals were regarded as "trepha" in contradistinction to certain portions of healthy animals fit for human consumption which were "kosher." But these inspections presuppose a fair knowledge of the normal and of some of the common diseases, and the writings in the Talmud indicate that the Jews possessed some knowledge of diseases of the lungs, liver, kidneys, intestine, mouth and other organs, although they shared many of the errors of the Babylonians and

their Egyptian contemporaries especially with reference to anatomy and physiology.

Egypt the land of hieroglyphics, has thus far revealed little advance in the knowledge of essential pathology. This is rather surprising since they practiced evisceration in the preparation of their mummies; further their papyri indicate a fair knowledge of many intestinal disorders especially the parasitic diseases. That some of our common diseases existed among the Egyptians has been definitely established by the recent identification of the pathologic lesions. Smith and Ruffer have described a case of tuberculosis of the spine in an Egyptian mummy whose age is estimated at 1000 B.C., but there is no evidence thus far that the Egyptians understood the nature of the disease. It remained for Hippocrates to associate this condition with pulmonary tuberculosis. Before him, however, Aesculapius, who tradition tells us was the son of Apollo, the Greek god of healing, had established temples managed by priest-physicians which approximated the modern health resort Prayers and sacrifices were followed by baths, massage, catharsis, blood letting and other regimen; and when the grateful patient was cured he presented an offering to the temple which frequently was a model of the diseased part in wax or precious metal and so began the inception of the pathologic museum; although Bass states that this custom also existed among the Egyptians.

Hippocrates who lived 460-370 B.C. was not only the greatest of Greek physicians, but probably the greatest physician of all time. More than any other individual he helped establish the identity of medicine as a science and dissociated it from philosophy and theology of his age. He was the first to establish a definite system of pathology in which he attributed all disease to disorders of the several fluids of the body. He believed that there were four essential humors or body fluids,—blood, phlegm, yellow bile and black bile; that as long as they remained in propor proportions health was retained, while alteration in the normal ratio was manifested by disease. This theory, of course, collapsed as progress in medicine was made; but its spirit persists in the modern humoral theory of the mechanism of resistance to infectious diseases, and it is only befitting that one of the greatest of modern physicians, Paul Erhlich, should be the exponent of this view. In addition, the writings of Hippocrates indicate a knowledge of tuberculosis, malaria, mumps, anthrax, puerperal septicemia and other conditions, although the observations are clinical rather than pathologic.

Galen lived in the second century of the Christian era. He combined the humoral theory of Hippocrates with the mathematical ideas of Pythagoras, reducing the whole structure of pathology to a series of exacting postulates in which the numeral four plays a most significant part. However, he gave us the four cardinal signs of inflammation—rubor, tumor, calor, dolor, according to Neuburger; also, he taught that there is no disturbance of function without organic lesion; further he was an uncompromising dogmatist, and prepared a huge encyclopedia of the medical knowledge of his day which for centuries afterwards was accepted as infallible. Galen

is generally cited as closing the period of antiquity which was followed by the Byzantine period which is conspicuous for its absence of progress in medicine. It appears as though the medical men of this age had been drugged by the galenicals of the previous era. Of interest is one Theophilus Protospatharius, a physician and a captain of the guard who wrote a treatise on the urine upholding Galen's view that the urine is derived from the blood being elaborated in the portal vein and vena cava.

The Byzantine period (476 to 732) is a cold-storage period in the history of medicine after which the leadership in medicine seems to have passed under Arabian and Jewish influence.

Rhazes who lived in the tenth century has given good descriptions of the contagious diseases, notably smallpox and measles, and Isaac ben Solomon, a contemporary of his, wrote a book on uroscopy which enjoyed a wide circulation for centuries. Another outstanding leader of this period is the Jewish physician, Avenozoar, who lived in the Western Caliphate in the twelfth century. He was a clear thinker and an independent observer. He wrote on pericarditis with effusion, mediastinal tumors, otitis media, among other things, also he first described accurately the itch mite and Garrison calls him the "first parasitologist after Alexander of Tralles."

During the Middle Ages (1096-1438) pathology along with the rest of medicine slumped into a sort of quackery. Hand in hand with the wandering lithotomist, the barber-surgeon and conjurer of spells went the uromancer or the charlatan who proposed to arrive at all sorts of inaccessible material concerning the patient by the inspection of the urine such as the chastity of a woman, the sex of the unborn child, the condition of the liver, kidneys, heart and other organs. Undoubtedly many of these men were sincere in their prognostications, believing that they were based on reason and fact. On the other hand, the writings of others indicate their frank admission of quackery. According to Hollander, one Villanovanus, a leader of the school at Montpellier, taught his students in the year 1300 to make a diagnosis of obstruction of the liver when inspection of the urine failed to suggest any other condition. He further cautioned them that should the patient complain of a headache to insist that the headache came from obstructions of the liver; further, it was well to use such words as obstruction which the patients did not understand for to do so was to create a favorable impression. If the physicians of this period made little advance in medicine it was not through lack of opportunity. At no other period were there as many widespread epidemics claiming such a fearful toll of human life. The Black Death claimed millions; syphilis, thought to have been introduced by Columbus on his return trip, swept Europe like a pestilential epidemic, influenza, leprosy, epidemic chorea, ergotism and the sweating sickness each claimed its toll. The most enlightening phase of medieval medicine is the establishment of hospitals and the beginning of organized nursing.

The year 1453 marks the beginning of the Renaissance in medicine. This is the period of the revival of learning and the Reformation. A number of agencies were at work whose resultant force lifted the pall of the

medieval period. Among these may be mentioned the invention of the printing press, and the discovery of gun powder with its application in firearms, the discovery of America, and perhaps the bursting forth of long suppressed longing for individualism as opposed to feudalism. The outstanding medical personage of this period is Paracelsus (1493-1541) also called Aureolus Theophrastus Bombastus von Hohenheim. He has been called the most original thinker of the sixteenth century. Having studied alchemy, astrology and other occult sciences, he traveled about hobnobbing with barbers, executioners, midwives and fortune tellers. Nevertheless, in spite of his low tastes for company he was appointed professor of medicine at Basel in 1527. He discarded Galenism and the four humors,—he opposed uromancy and witchcraft,—he wrote on occupational diseases and correlated endemic goitre with cretinism and contributed a number of drugs which today are found in all pharmacopeias. Among his contemporaries were some famous anatomists who lifted this study literally out of the mire of public contempt and made it a living working science. Among these were Leonardo da Vinci, better known as a painter, Vesalius, the author of the epoch making "De Fabrica Humani Corporis" (1543), Sylvius, Fallopius, Eustachius, and others whose names are identified with our anatomical nomenclature.

One of the great leaders of this age was Ambroise Paré who contributed materially to the advance of anatomy, pathology, medicine and surgery. He popularized pathology in France by his numerous postmortem examinations including the bodies of persons of high rank among whom were no less than three kings, Henry II, who died following a lance thrust over the right eye which penetrated the brain, accidentally incurred at a court joust; the King of Navarre, who died from infection following a gunshot wound received during the Siege of Rouen; and Charles IX, who died peacefully from phthisis. Like Paracelsus, who discarded the classic languages and wrote in his native German, so Paré wrote in his native French. Born of humble parents he rose to the position of the first surgeon to the king. Near the end of his career he was the victim of a series of quarrels between the physicians, surgeons and barber surgeons of his day, necessitating his addressing a pamphlet to the Parliament of Paris in justification of himself and his work.

The seventeenth century is rich in the history of medical achievement. Harvey demonstrated the circulation of the blood and put physiology on a new basis. The microscope was coming into use, and enabled the investigators to study the finer structures of living matter which was destined to culminate in the enunciation of the cell theory two centuries later with the subsequent proclamation of the identity of protoplasm in all forms of life, both animal and plant.

One of the earliest microscopists was the Jesuit priest Kircher. This is of special historical significance in view of the muchly heaped abuse on the Roman Catholic Church, which has been accused of impeding medical progress. The sometimes quoted ecclesiastic bulls were in fact proclaimed

with the intent of protecting the bodies of dead soldiers from desecration. Kircher was probably the first to employ the microscope in the study of disease. He attempted to study the nature of the process of putrefaction by action of maggots in decaying matter; also, he made some observations on the blood of plague victims. A contemporary of his, Malpighi, has been called the greatest of microscopists and the founder of histology; he investigated the embryology of the chick and the histology of glands and viscera; he described the red blood corpuscles. His name is preserved in the Malpighian layer of the skin and the Malpighian corpuscles of the spleen and kidney. Redi opposed the view of spontaneous generation by showing that maggots did not appear in decaying meat when protected by wire gauze. Peyer described the hyperplastic intestinal glands of typhoid fever which bear his name; and chemistry was beginning to emerge from alchemy and the black art. Potable gold, the elixer of life, and the philosopher's stone had had their day and were being discarded. Physician and chemist were seeking an explanation of respiration which was to culminate in the discovery of oxygen the following century. Many important monographs appeared in clinical medicine and pathology, including observations on typhus fever, scrofula, scurvy, malaria, dysentery, rickets, ergotism, cretinism, apoplexy, glanders, pulmonary emphysema, asthma, angina pectoris, lead colic and numerous others. Of special interest is the first detection of albumin in the urine by Dekker of Leyden made by boiling urine in the presence of acetic acid, although glycosuria had been known almost from the inception of ancient medicine and had been described as "honey urine" by the early Hindus.

Uroscopy was falling into decadence but nevertheless was still a favorite theme of the artists who have preserved for us the visual impressions of the physicians of their day. Hollander describes a famous painting by Gerard Dous, a pupil of Rembrandt, in which a dignified old prosperous physician stands in a grey cape fringed with violet at an open gable window which is half covered by a Brussels carpet and through which light streams into the room from the side disappearing in a toned chiaroscuro. On the balustrade before him is a collection of articles connected with the profession of the healing craft including a parchment diploma with the seal of the university, a tin basin, a copper morter, a globe, a folio volume, a viol, a candle, the beloved skull and the typical container of minor surgical instruments, while in the middle of the room is suspended a cupid as the guardian angel. The physician himself holds the glass flask in his hand inspecting the reddish urine which an old woman has brought. The eyes of both are directed to the test and in both there is an expression of sad resignation as the physician contemplates the specimen.

The eighteenth century is rather disappointing for its failure to keep pace set for its progress by the brilliant start of the previous century. There is something suggestive of feudalism attempting to assume its former influence, if not over the bodies of men, at least over their minds by systematizing all new thought into well regulated and organized classifications.

In spite of this inauspicious spirit of the time, clinical pathology made considerable progress. In fact aside from the Linnaeus' classification in botany, clinical pathology may claim practically all the significant observations of this century as occurring in its domain. In chemistry carbon dioxide was discovered by Black in 1757, hydrogen by Cavendish in 1766, nitrogen by Rutherford in 1772, chlorine by Scheele in 1774, oxygen by Priestley in 1771 and Lavoisier in 1775, and the composition of water by Cavendish in 1781.

In pathology, postmortem examinations were being introduced into clinics and a systematic attempt was being made to correlate the observed anatomical alteration with the clinical symptomatology; in serology observations were made on the coagulability of the blood by Hewson with reference to experimental delay and search was begun for the property of the blood causing coagulation, later to be identified as fibrinogen; and in bacteriology Jenner's work with preventive inoculation for smallpox was not only the great triumph of the age, but one of the greatest of all time. Beginning with the country-side yarn that milkmaids who contracted cowpox were immune from smallpox, Jenner attempted to transfer the immunity from a milkmaid to a boy by inoculating him with the matter expressed from a discharging lesion of the maid ill with the cowpox. When the boy was subsequently exposed to smallpox he was found to be entirely immune to it. Having collected 23 cases, Jenner published them in 1798 in a thin quarto under the title of "On Inquiry into the Cause and Effects of the Variolae Vaccinae."

The nineteenth century begins the modern period in medicine. Just as in the sixteenth century we find the great anatomists whose names have been incorporated into the anatomical nomenclature so we encounter numerous physicians in this era who described certain pathological conditions and whose names are now associated with them. Among them are Laennec, Graves, Stokes, Bright, Corrigan, Addison, Hodgkin, Parkinson, Jackson, Dupuytren, von Graefe and numerous others.

The cell theory was enunciated and established; pathology was placed on a cellular basis; chemistry witnessed the rapid discovery and isolation of numerous elements; the atomic and periodic tables were formulated; organic chemistry came into existence to be followed rapidly by physiological chemistry which paved the way for the newer blood chemistry; many of the infectious diseases were analyzed and the etiological factors demonstrated and the science of bacteriology was established; the numerous phenomena of immunity and serology were demonstrated and complement fixation was made a working principle; laboratory tests passed through a rapid succession of improvement, from the qualitative to the quantitative, and from the quantitative to the functional introducing the time concept into diagnostic methods and finally the more intricate laboratory tests as basal metabolism was introduced, the proper performance and interpretation of which requires a working knowledge of mathematics, physics, chemistry, pathology and clinical medicine. In fact so numerous and voluminous

were the significant discoveries that in a brief paper of this kind we can enumerate only a few of the more significant achievements.

Following the work of Kircher, Hooke, Malpighi, Grew, and others who made observations with the simple and imperfect microscope of the day, the early microscopists studying plant tissues saw small compartment-like spaces surrounded by a distinct wall filled with what they thought to be air or fluid and to which the name "cell" was applied. In 1831 Robert Brown directed attention to a small body found in the cell to which was given the name "nucleus" and in 1836 Valetin described the nucleolus within the nucleus. In 1838 Schlein proclaimed and demonstrated that plants were made wholly of cells and in 1839 Schwann declared that the animal body the same as plants was made up wholly of cells; in 1846 Mohl recognized the semifluid granular substance within the cell which he named protoplasm; in 1855 Virchow announced his famous aphorism "omnis cellula e cellula" and in 1861 Schultz proclaimed the identity of protoplasm in all forms of plant and animal life.

Virchow has been called the greatest of pathologists. In 1847 he founded the "Archiv für pathologische Anatomie" generally known as Virchow's Archiv; in 1856, he was appointed professor of pathology at the University of Berlin and in 1858 he published his "Cellular-Pathologie" in which he attacked the theory that there existed an invisible vital force distributed through the entire body or located within a few organs, instead this force was subdivided into an infinite number of individual associated vital forces which were located in the individual cells. "The cell," he wrote, "is actually the ultimate proper morphological element of every vital manifestation." He expressed the view that the body was a cell state in which every cell was a citizen and that disease was a conflict of the citizens within the state brought about by external forces, further he discredited the old view that there were distinct specific disease cells but regarded inflammatory and tumor cells as modifications of physiologic types. He wrote on a multiplicity of pathological conditions besides on archeology, anthropology, epidemiology and social conditions of his day. "The cellular theory," says Baas, "like all theories is to be regarded as a historical expression of the scientific tendency of a certain period."

In bacteriology perhaps the earliest observation was made by the Dutch microscopist, Anton van Leeuwenhoek, in 1683, who described the motility of organisms in scrapings from teeth; later O. F. Muller, in 1786, succeeded in discovering many structural details of microorganisms and gave descriptions of several kinds of bacteria, but it remained for Pasteur to establish the science of bacteriology. His researches in fermentation and spontaneous generation imparted to the study of bacteria a broad biologic importance. Pasteur demonstrated that putrefaction and decay which had been ascribed to spontaneous generation were caused by clinical disintegration of organic material due to the metabolic activities of bacteria whose primary interest was their search for food.

Koch was, perhaps, the first to show a specific relationship between a

bacterium and a disease. In 1876, he brought forth convincing proof that the bacillus anthracis was the cause of anthrax in cattle. In 1883, he discovered the cholera vibrio, and in the same year he described the organism of infectious conjunctivitis, now known as the Koch-Week's bacillus. He formulated "Koch's postulates" which prescribes the requirements for establishing the specificity of a casual microorganism to its disease.

He established definitely the etiology of tuberculosis although before him, in 1865, Villemin had shown that this disease was infectious. In 1871-4 Hansen established the causal relationship between leprosy and its organism; in 1879 Neisser called attention to the constant presence of a peculiar coccus in gonorrheal pus; in 1880 Pasteur observed staphylococci in pus from suppurative processes and in the following year Ogston described streptococci in inflammatory exudates; in 1883 Klebs described the diphtheria bacillus and in 1884 Loeffler emphasized its causal relation to the disease; in 1884 Frankel discovered the pneumococcus and in 1885 Leichtenstein saw the intracellular diplococcus in meningeal exudates; these were followed by numerous other significant discoveries including the organisms of glanders, erysipilas, tetanus, Malta fever, bubonic plague, puerperal infections and numerous others, and more recently, Hoffman and Schaudin in 1905 discovered the Spirocheta pallida.

In serology Brieger first isolated toxins in 1888, and in the following year Buchner demonstrated the bactericidal power of blood serum; later he showed that this property existed in the serum. In 1890, Behring and Kitasato demonstrated that rabbits and mice could be artificially immunized against tetanus and that this immunity was associated with the ability of the blood plasma to render harmless the toxic substance produced by the tetanus bacillus which substance they called "antitoxin," previously, in 1888, Nuttall showed that blood loses its bactericidal power when heated to 56° for one-half hour; in 1894, Pfeiffer demonstrated bacteriolysis in vivo, and in the following year Metchnikoff and Bordet demonstrated that this lysis occurred in the test tube if the organisms were mixed with the serum of an animal previously injected with the organisms.

In 1896, Pfaundler drew attention to the peculiar phenomenon of agglutination which occurred when bacteria were grown on immune serum and in the same year Widal and Grunbaum turned these facts to practical use in the diagnosis of typhoid fever. In 1897 Kraus described the bacterial precipitins—he observed that when the serums of animals that had been immunized against specific organisms were added to a clear filtrate of the respective bouillon cultures of their bacteria, the clear solution became turbid and a precipitate formed. Nuttall in a very painstaking research showed that the blood of practically every animal could be identified by its specific precipitin reaction, also, he showed the blood relationship of various animals based upon group precipitins.

In 1898, Bordet demonstrated that red blood cells would undergo lysis both in animals and in the test tube provided the animals in which the red cells were injected or from which the serum was drawn, had previously

been inoculated with homologous red cells; further he demonstrated that three constituents were necessary—the specific red cell, the serum of the animal injected with these and a constituent of all normal serum discovered by Buchner and called alexin by him and now called complement. Neisser and Wechsberg, in 1901, showed that complement not only acts quantitatively in being fixed but that it can be deviated. Upon these principles the complement-fixation test developed with bacterial suspensions used as the first antigens, later to be replaced by various fractional modified extracts.

In chemistry, the nineteenth century witnessed enormous progress. It is reasonably safe to state that in this century more progress was made in this science than in all previous time, and the twentieth bids fair to eclipse the nineteenth. It had been taught for many centuries that matter was made up of small indivisible particles, but it remained for Dalton to propound a truly chemical atomic theory, declaring that the atoms of the elements are not of the same weight and that the relative atomic weights of the elements are the proportions by weight in which the elements combine. In 1803, he published his first table of atomic weights bearing out this view. The atomic values given by Dalton have since been shown to be incorrect, but he paved the way for others. In 1818 Berzelius announced his theory of chemical proportions and made use of chemical symbols and formulae as they are employed today. Previously in 1809 Davy described the nature of alkalies and Gay-Lussac described the law regulating the combination of gases by volume, and Avagodro, in 1811, declared the law that bears his name that under constant pressure and temperature, the number of molecules of a gas in a given space is always the same. In organic chemistry, Wöhler, in 1828, prepared synthetically urea from inorganic constituents and bridged the gap between the inorganic and organic substance, and later, he showed that benzoic acid taken by mouth appears in the urine as hippuric acid. This experiment is sometimes cited as the beginning of the chemistry of metabolism. A few years earlier Prout, in 1824, demonstrated that the essential acid of the gastric juice was hydrochloric acid, Liebig, a pupil of Gay-Lussac, helped to establish the science of organic chemistry by his studies of cyanides, cyanates, hippuric acid, chloral, chloroform, amides, aldehydes and numerous others. Synthetic organic chemistry made enormous strides during this century and one of the greatest contributors to this branch was Fischer.

The study of chemical metabolism of the body was developed and threw great light on the diverse functions of the body in health and disease, especially the constitutional disorders; yet there were many problems that were unexplained. The science of nutrition was greatly facilitated by the discovery of the isodynamic law which states that a given amount of food if taken into the body may liberate practically the same amount of energy that it would produce if burned in oxygen outside the body; this permitted the application of the measurement of food by a method similar to the method used in measuring the energy derived from coal. An important contribution was made in 1906 by Hopkins who observed that growth of the body required something in addition to the established requirements of carbohydrates, pro-

teins, fats, salts, water and oxygen. He called them hypothetical substances "accessory food factors" and in 1911 Funk coined the word "vitamines." The study of vitamines has thrown light on such conditions as scurvy, beriberi, rickets, and other conditions.

The development of colorimetry enabled the quantitative estimations of substances and amounts heretofore regarded as impractical of measurement by the chemical balance and volumetric methods, and hand in hand with this new system of chemical analysis went the development of the newer methods of blood chemistry as the quantitative determination of blood sugar, nonprotein, nitrogen, creatinin, uric acid, urea and other substances. It is significant to us that most of the observations in this branch were made by American observers. Prominent are the names of Folin, Benedict, Van Slyke, Meyer and others. The information which these blood chemical methods have rendered available is especially important since the older method of examination of these conditions by cytology, bacteriology and serolgy gave insufficient information. They have been especially helpful in the analysis of the constitutional diseases as diabetes mellitus, nephritis, gout, renal diabetes, etc.

Clinical laboratory methods went through a rapid succession of improvements. Emerging from the old art of uroscopy which made use of inspection primarily, chemical reactions came into use and established definitely the presence or absence of certain substances.

In 1848, Fehling published his quantitative test for sugar in the urine. This test and its modifications have acquired a time honored place in the laboratory because of the ease of manipulation and definiteness of results and it is probable it will continue to remain one of the commonly employed methods. Qualitative data were improved when quantitative methods were introduced because knowledge of the amount of sugar gives greater information of the disease than merely information as to the presence of sugar. A quantitative test of the amount of sugar in the blood is still more valuable because it approaches closer to the site of the pathological lesion and a functional sugar tolerance test such as recommended by Haman and Hirschman represents the highest type of biochemical test so far introduced because it measures the working capacity of the body to utilize the sugar in question.

The significant feature of the functional test is the introduction of the time concept into laboratory methods. The functional test is always made with reference to the time of something else such as the time of taking of food, the time of administration of drugs, the time of performing exercise, etc. In this respect the functional test is in conformity with Einstein's theory of relativity which links up the time element as a sort of fourth dimension to our previous three dimensional concept; and with the introduction of the time element certain standards previously considered absolute now become relative; for instance, 0.14 per cent blood sugar may be normal or it may be pathological depending upon its relationship to the time of taking of food. If the test is made within two hours of food taking, it probably is normal, but if it occurs twelve hours after food taking it is probably pathological.

The same relativity holds true in the other biochemical functional test. The single gastric analysis is no longer regarded as satisfactory. According to the observations of Hawk, Rehfus and Bergheim, it is unsatisfactory to interpret the data obtained from the examination of the single test meal removed at one hour interval as this represents but one phase in a constantly changing cycle of gastric digestion and it is impossible to judge what has preceded or what has followed. A given figure may be a part of normal, increased or decreased gastric curve.

In this paper there has been no attempt to trace the evolution of pathology itself but only the evolution of laboratory methods. Disease existed in earliest primitive man and before him in the vertebrate mammals, and before them in the earliest organisms that lived countless millions of years ago. Bacteria existed in early pre-Cambrian era and the fossil remains of nitrifying coccoid organisms have been found in the mountains of Montana by Wolcott, the age of these mountains being estimated at 32,000,000 years, but the exact knowledge of bacteria may be said to have begun with Pasteur a few decades ago.

Finally it appears that history must repeat itself. Just as we have read of the wrangles between the barbers, the barber-surgeons, the surgeons and the physicians of a past age each trying to maintain its caste, so we encounter the comments of a few self-appointed censors today. In the December 2, 1922, issue of the official journal of our good mentor the American Medical Association the statement is made by some nameless individual who hides his identity under the cloak of the Advisory Advertising Committee of the Board of Trustees of this Association and writing anonymously states that "the status of the clinical pathologist is not the same as that of the internist or surgeon. The latter deals with variables—human beings—the former conducts manipulations on fixtures—inanimate substances." This writer evidently was thinking of the famous painting by Dous showing the physician of a past age inspecting the urine. One is inclined to wonder whether he ever saw a basal metabolism test performed, or a functional sugar determination requiring the removal of a sample of blood every half hour for three hours, or a fractional gastric analysis or any other of a multiplicity of tests requiring very exacting control of the patient.

The clinical pathologist differs from the internist it is true but not according to the standard mentioned by the nameless one. The clinical pathologist does not treat disease, he confines himself to the performance of the more exacting diagnostic measures.

If any lesson is to be learned from the history of medicine, it is that one of the first duties of the physician is to understand the nature of disease, and in this respect the clinical pathologist functions as a true physician.

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THE EFFICIENCY OF RADIATION THERAPY*

By U. V. Portmann, M.D., Cleveland, O.

SINCE the advocation and development of deep x-ray therapy by German specialists, roentgenologists in the United States have eagerly accepted their teachings and with characteristic enthusiasm have begun the treatment of all types of malignant conditions. Some of our best qualified roentgenologists have investigated the methods employed in European Clinics and have indorsed them with qualifications, which to a certain extent have been overlooked by many who are now attempting to employ deep x-ray therapy. It was because of our inexperience in the field of deep x-ray therapy that it became necessary to accept the work of European physicists and other investigators as the primary basis for the work in America. But unfortunately the methods employed in Europe are not entirely adaptable to our own different working conditions. In particular our American apparatus for the measurement of intensity and our generating apparatus are not the same as theirs. The American manufacturers of x-ray apparatus have made an attempt to meet the demand by marketing high voltage machines, with a resultant unfortunate competition and commercialism of a valuable but dangerous weapon.

We found that European methods were not always applicable to conditions here, but European physicists are now revising and retracting some of their earlier work so that now their results more nearly conform with later results of experiments performed by Americans.

In our own Clinic the experimental application of physical principles to

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the work of the x-ray department is conducted by a physicist, Hugo Fricke, Ph.D., who very early called our attention to certain discrepancies between our results and those first reported from Europe. Dr. Fricke uses an electroscope of his own design made in the mechanical department of the Clinic. This is well protected from possible sources of error. The ionization chamber is of organic substance and its material and design were chosen after considerable study and experimentation. The investigation was conducted under our own working conditions with standard American apparatus. A room adjoining the therapy department made it possible to conduct the experimental work under conditions identical with those under which the therapy would be applied. All factors were checked by approved methods of measurement.

Very many factors enter into the correct interpretation of measurements made by the ionization method. Dr. Fricke carefully studied the published reports and found in them certain errors which were corrected in our own laboratory so that now we feel that our work is as exact as is possible. For example, as our work progressed we learned that our equal intensity curves though almost similar in configuration gave much less of a depth dose (10 cm.) than those published. In fact, we were unable to obtain a depth dose approaching 34 per cent under any practical working conditions. Since determining our own intensity curves we have found that other workers have come to a similar conclusion regarding the earlier reports: that is, that the depth of x-rays under practical working conditions is considerably smaller than was originally determined. The results of Dr. Fricke's work are to be published at an early date.

When applying our equal intensity absorption curves in the treatment of patients we found it impossible by any method of application to administer a deep "carcinoma" which I call a therapeutic dose, or say a 120 per cent dose, to an average case such as an uterine carcinoma. We found that in such a case not less than five portals of entry as large as 10 by 15 cm. each are necessary to deliver a deep dose of from 85 to 100 per cent. In increasing the size of portal to more than 20 × 20 cm. very little difference in depth dose results and the larger size is impracticable. Even if we use factors giving the maximum or 34 per cent dose, on account of dosage received at opposite or neighboring portals the total dose delivered at one or more portals must be diminished, thus losing a percentage equal to the amount of that diminution at the required depth.

If the conclusions of the physicists are correct and the many reports of apparent cures are well-founded, we must conclude that deeply situated pathologic lesions yield to a smaller dose of radiation than has previously been supposed. But it is highly improbable that deeply situated carcinoma yields to a smaller dose than does superficial carcinoma and we know that the cure of most malignant growths requires a very large dose of radiation. If radiation therapy has any place in the treatment of malignancy and we know it has, it must be given in large doses at one time. It is possible, therefore, that some of the apparent cures reported from x-ray therapy alone, have

really had insufficient radiation and later disappointment in the results of deep x-ray therapy will develop.

For ourselves, as the result of our short, practical experience and from the findings in our experimental investigation, we believe that with our present methods it is impossible to give a sufficient therapeutic application of x-ray radiation to most deeply situated malignancies in one dose. But we have learned also the inadvisability and danger of insufficient radiation and of repeated doses of x-ray.

It is absolutely essential that each case be thoroughly studied not only as a biologic but as a physical problem, i.e., the physical laws of radiation must be properly applied to each individual case. The average roentgenologist is poorly equipped to make the essential studies of the physical laws which are involved; he has not the time, the apparatus, or the special knowledge, each of which is necessary. We believe that the empirical application of x-ray radiation to patients with malignant diseases is detrimental—some apparently good results notwithstanding. There is no question as to the value of radiation therapy—the only question is as to the proper method of application.

Fortunately in any case in which the radiation which can be applied to a malignant tumor is therapeutically insufficient the valuable assistance of radium can be employed to supply the percentage of the dose that is lacking. Dr. Henry Schmitz and others have frequently called attention to the combined method of radiation. Even with the quantities of radium ordinarily available it is possible to administer a therapeutic dose of radiation if the radium and x-ray intensity curves are understood and properly applied. By superimposing radium intensity curves over x-ray intensity curves through the necessary portals of entry, we are able to build up an evenly distributed and homogeneous therapeutic dose of radiation not only to the tumor itself but also to distant areas that may or may not be malignant. Thus we approach the technic of the surgeon who has learned the necessity not only of removing malignant tumors, but of making also a wide-spread dissection. It is probable that those who rely upon either x-rays or radium alone, will be very much disappointed in the ultimate results. Moreover we are not yet ready to state that there is no specific cure for certain malignant diseases in x-ray or radium. Our best weapon is the judicious combination of surgery, radium and the x-rays in the proper proportions for each individual case. It should be borne in mind that while surgery seems to have rached the limits of its application to the treatment of malignant diseases, the use of radiation is still in its infancy. The value of radiation has been demonstrated, but in the present stage of our knowledge we cannot foresee what may be its final potency. Until that limit has been demonstrated we must be prepared to use every available weapon in our attack upon malignant tumors.

The problem of radiation therapy is to determine and administer a maximum therapeutic dose of x-rays in combination with a proper dose of radium, and in making this determination it must be constantly borne in mind that while the x-ray radiation is diffuse, the sphere of radium radiation is within this area; therefore, care must be taken that in the resultant overlapping of

the maximum intensities of each the surrounding normal structures are not permanently damaged. Radium may be applied by whatever methods are preferred by the radiologist, but the equal intensity curve for whatever method is employed should be understood. In our Clinic the radium is applied by Dr. T. E. Jones who prefers to use rather large quantities of radium and arranges the needles with great care so as to obtain a widely homogeneous radiation of distant pathologic areas. The x-ray radiation is administered in an intensity sufficient to build up an even therapeutic dosage at least 10 cm. from the point at which the radium is inserted.

Unfortunate results of overradiation by deep x-rays have been reported, but these must be due to too frequently repeated treatment. The danger of insufficient radiation is almost as great. In our experience those cases which were not properly irradiated at the first treatment did not do well following subsequent treatments and the end results have not been as good as when a properly measured dosage was applied at the first radiation. Damage to the adrenals has been reported. Whether or not radiation produces fibrosis of the lung is unsettled. Some workers state that fibrosis of the lung has not occurred in their experience; but we have observed several cases in which it did follow full doses with only one-half millimeter of copper filter. On enquiry several roentgenologists state that they have lost cases from afebrile "pneumonia" about six weeks after radiation which is the period of time after which we have observed the fibrosis to develop. It may be that the lighting up of a latent infection is responsible for this condition. On the other hand we have subjected tumors of the lung to radiation without subsequent ill results.

SUMMARY AND CONCLUSION

From our study and experience we conclude that:

(1) In order to obtain the best results in the treatment of malignant diseases by radiation the physical principles of the production and action of the x-rays and of radium must be understood.

(2) It is usually impossible to deliver a therapeutic dose of x-ray radiation to deeply situated areas. The large deep doses which were first reported are not obtainable under present practical working conditions.

(3) Radiation by either the x-rays or radium alone is insufficient; they should be used in proper combination.

(4) The danger of insufficient radiation is as great or greater than that of overradiation; the desideratum is the administration of the proper dose at the first treatment.

BACTERIOPHAGE PHENOMENA. I*

LLOYD ARNOLD, M.D., CHICAGO, ILL.

DURING the past five years there have been a great many publications dealing with the "bacteriophage phenomena." We do not wish to go into the literature on this subject, as this has been already reviewed by several authors in this country, Davison,[10] Kuttner,[4] d'Herelle-Smith.[5] We wish only briefly to mention the methods that several workers have used in obtaining the "bacteriophage substance."

D'Herelle[1] inoculated bouillon with the fecal material from a patient convalescing from dysentery (B. dysenteriae shiga). After 18 hours incubation this was passed through a Chamberland filter. Several drops of this filtrate was added to a young bouillon culture of B. Shiga, causing a complete clearing of the tube after a short period of incubation. A suspension of B. shiga, obtained from a young agar culture, added to this cleared tube, and incubated overnight, again became clear. One drop of this cleared or lysed culture was sufficient to cause complete clearing of a young culture of B. shiga. This could be transmitted in series.

Bordet and Ciuca[2] showed that this same "bacteriophage substance" could be obtained from other sources than the stool filtrate. These workers injected a strain of B. coli into a guinea pig intraperitoneally, three or four times at intervals of a few days. The exudate so obtained was diluted with broth and allowed to stand at room temperature for a day or so and then filtered. This filtrate when added to a normal culture of the B. coli produced lysis transmissible in series.

Kuttner[3, 4] obtained a "bacteriophage substance" from glycerol extracts of the intestine, and the acetone precipitate of the liver of guinea pigs, also from normal rabbit serum.

Some time ago we found that the washed suspension of the surface of an agar culture of irregular or bacteriophage colonies when passed through the Berkefeld filter, contained a strong bacteriolytic substance.

We found that if the whole of the agar, after the surface lycogenic growth had been washed off, was transferred to a flask and shaken with either distilled water, normal salt solution, or bouillon, and then passed through a Berkefeld candle, this filtrate showed a stronger bacteriolytic activity than the surface washings. Both of these filtrates cause lysis transmissable in series and did not differ in any way from the bouillon filtrate prepared by the d'Herelle method in so far as the "bacteriophage phenomena" was concerned.

*Department of Bacteriology, Pathology and Preventive Medicine, Loyola University School of Medicine, Chicago.

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There was a large amount of protein material in the bouillon in addition to the biproducts of metabolism of the young culture of bacteria and when cleared by the d'Herelle method, the bacterial protein content must be considerably increased. The washings of the surface growth of the lycogenic culture contained bacterial proteins. The agar, remaining after this washing, when extracted with water contained some bacterial proteins and also the bouillon base used in the agar preparation. We found that we could repeatedly extract this agar and each time remove in the extracted aqueous medium some bacteriolytic substance. The first extract was bouillon colored, the second a trace of this coloring, the third was usually water-clear, the fourth and remaining extracts were always clear. We have extracted the same agar as many as twelve times, and even in the twelfth extracts found a "bacteriophage substance."

For some of the experiments to be reported in this paper, and for a future publication dealing with some questions of immunology, we have used the following method for the preparation of our "bacteriophage" or bacteriolytic principle.

Washed and dried agar was added to distilled water to make a 2 per cent concentration. This was heated until dissolved and filtered, then autoclaved in convenient sized flasks. Ten c.c. of this water-agar was poured into a sterile petri dish. After cooling a piece of thin sterile tissue paper, 0.5 mm. less in diameter than the inside measurement of the dish, was laid on top of this sterile water-agar layer. Then 7 or 8 c.c. of standard nutrient agar was poured on top of this tissue paper.

The surface of this nutrient agar was seeded with a lycogenic strain of bacteria and incubated for twenty-four hours. By means of sterile forceps the tissue paper with the nutrient agar layer can be removed as one piece without contaminating the under layer of water-agar. This lower layer is then removed, pressed through an ordinary coffee sieve to break it up into small particles. Ten c.c. of water is added for each agar plate used, also a little toluol, and put in the shaking machine for 8 to 12 hours, then in the ice box overnight. The fluid is strained off through glass wool and a coffee sieve, filtered through wet filter paper to remove the toluol, and then passed through the Berkefeld filter. The agar is returned to the flask, and equal amount of water is added, shaken, etc., repeated.

The filtrates obtained from these bottom layer water-agar extracts contained a bacteriophage substance, transmissible in series, inactivated at 75° C. and conformed in every way with the original d'Herelle bacteriophage. The fourth extract is water clear, the first and second contain bouillon absorbed from the upper layer. The third extract is sometimes bouillon tinged in color.

The extracts are active up to the twelfth one, after this there was only a trace of bacteriophage present and after the fifteenth extract the bacteriophage activity was negative.

D'Herelle found that the precipitate formed upon the addition of 9 parts of alcohol contains a substance causing lysis, but this was not trans-

missible in series, and he regarded this to be the "lysin," the active principle extracted from the "bacteriophage." The precipitate formed upon the addition of 9 parts of alcohol to either of the first three extracts does not contain any substance causing lysis. Upon the addition of alcohol to the fourth and remaining filtrates there is no precipitate formed, although they contain a very active bacteriolytic agent.

We have run controls in the following manner: The bottom layer of water-agar was extracted after incubation without inoculation, and also extracted after the upper nutrient agar layer had been inoculated with B. typhosus (Olsen). In neither instance was there a bacteriolytic substance obtainable.

The original source of the bacteriophage used by us was isolated from the stool of a case of typhoid fever by d'Herelle's method. This was actively bacteriolytic against several strains of B. typhosus and also against 8 of our 12 strains of B. dysenteriae. This will be referred to as B.S. in this page.

Feces of a normal two and one-half year old baby yielded a good bacteriophage against several strains of B. dysenteriae (Shiga, Flexner, Hiss, Sonne B, Strong). This will be referred to as B.B. in the future. (This bacteriophage and also the Olsen strain of B. typhosus was kindly furnished us by Dr. Kuttner.) The Olsen bacteriophage was used by us before we had the opportunity of isolating any of this substance for ourselves. This was designated as our B.Z. strain of bacteriophage.

EXPERIMENTS

Six tubes of bouillon, each containing 10 c.c., were inoculated with a loop of an 18-hour old bouillon culture of B. typhosus (Olsen). One-half c.c. of the B.Z. bacteriophage was added to each of 5 of these tubes. After thorough shaking, a loop of the first tube was seeded on the surface of a sterile agar plate and smeared well with a bent glass spreader; then the remainder of the contents of this tube was immediately passed through a Berkefeld filter. The other 4 tubes were placed in the incubator. At intervals of three hours, plates were inoculated and the contents of the respective tubes were immediately filtered as was done with the first tube. The remaining tube was plated out every three hours as a control for the relative amount of growth. The plates were incubated 24 hours. The ratio of the regular to the irregular colonies was recorded.

The plate from the first tube showed the ratio of the regular : irregular colonies as 80:20, second plate 5:95, third plate 80:20, fourth and fifth plates were regular colonies throughout. The filtrates from the respective tubes were pipetted into freshly inoculated bouillon cultures of several members of the typhoid-dysentery group to the concentration of 1:10, 4 strains of B. typhosus, 3 strains of B. dysenteriae (Flexner), 2 strains of B. dysenteriae (Shiga), 1 strain each of Strong, Hiss Y and Sonne B. were used. Filtrates from tubes 1 and 3 showed inhibition in only one typhoid strain (Olsen), one Flexner, and in Hiss and Sonne B. for 18 hours, after this growth appeared and remained cloudy for the period of observation (21 days). The filtrate 2, that is the 3 hour growth tube, inhibited all the strains used for 18 hours.

Of the typhoid strains, the Rawlings' and Hopkins' strains showed growth after 36 hours, the Olsen and Mt. Sinai after 96 hours. Two Flexner, one Shiga, the Strong, Hiss and Sonne B. strains were inhibited during the period of observation. The remaining strains showed growth either after 36 or 48 hours. The filtrates from tubes 4 and 5 showed an inhibition of the Olsen typhoid strain for 18 hours. All the remaining tubes were as cloudy as the controls.

This experiment has been repeated dozens of times, using hourly plating and filtrates instead of every three hours. We have used the B.S. and B.B. bacteriophages, the surface washings of plates showing irregular colonies, the agar extracts of these plates, and also the bottom-layer-agar extract. The results usually agree with these mentioned above. Some few variations occur and these will be treated more in detail later.

The B.B. bacteriophage, isolated originally from a healthy baby's stool, was usually active against B. dysenteriae (Flexner, Hiss, Strong and Shiga), in the order named. This strain of bacteriophage was not active against any of the strains of B. typhosus ordinarily, but the 3 hour bouillon filtrate of this bacteriophage with a susceptible dysentery strain was active against two of the four B. typhosus strains. d'Herelle,[5] Gratia,[6] Bail,[7] Doerr[8] and others have reported an increased production of the bacteriophage, upon incubation with a susceptible strain of bacteria, after the second or third hour. They mentioned an increased virulency of the bacteriophage for the susceptible strain of bacteria used. We have found this to be true and accompanying this is a bacteriolytic action against stains of the whole typhoid-dysentery group. We have not found any of these bacteriophages, even at their maximum virulency, to be active against gram-positive bacilli or cocci.

We have found that there is a parallel between the relation of regular to irregular colonies on an agar plate seeded from a bouillon culture containing a susceptible strain of bacteria with a bacteriophage and the virulency of the bacteriolytic substance in the tube at the time of seeding. As the irregular colonies increased in number the virulency or activity of the bacteriophage, at the time the agar plate was inoculated, was found to be increased. Twelve tubes of bouillon were seeded with B. typhosus (Olsen). To one tube a bacteriophage was added in the concentration of 1:10 and an agar plate was inoculated from this culture, before and immediately after the bacteriophage was added. After one hour's incubation, bacteriophage was added to another tube and plates inoculated before and after the addition, as was done in the first instance. This was repeated each hour for ten hours. In this way we added bacteriophage to cultures of a susceptible microorganism at the time of inoculation and at each hour up to and including the tenth hour of growth. These plates were incubated 24 hours and the character of growth recorded. Plates were again inoculated from these same tubes after 24 hours, 48 hours, 14 and 25 days. The results obtained from this experiment were more variable than those of the former one mentioned. We wish to give the average result obtained from 46 such experiments using a bottom-agar-layers of the B.S. bacteriophage.

The first plate, that was inoculated from the tube of bouillon seeded

with B. typhosus and the bacteriophage added immediately, did not contain many irregular or lycogenic colonies, seldom over 10 to 20 per cent. The second plate, seeded from a tube after one hour's incubation and the bacteriophage added, showed more irregular colonies, usually 25 to 35 per cent. The third plate showed that about 50 per cent of the colonies were irregular; the fourth plate, about 80 to 90 per cent were irregular colonies; the fifth plate, that is after 4 hours' growth and the bacteriophage added at this time, was usually sterile. The next three plates were usually either sterile or showed a high percentage of irregular colonies. The remaining plates usually showed good, heavy growths; the irregularity observed in these plates corresponds to the "appearances" described by American and English workers; "tàches vièges" by the French and "Loecher" by the Germans, in this field. The ratio between these clear areas, or "appearances," and the regular heavy growth was somewhat variable, the regular colonies usually predominated.

Inasmuch as we have observed the gradual increase in the number of

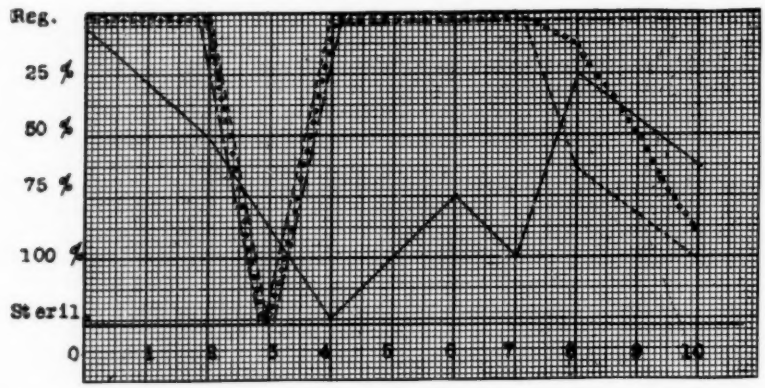

Fig. 1.—Abscissa is the age of the B. typhosus (Olsen) in hours at the time the bacteriophage was added.

Ordinate is the percentage of irregular colonies on the agar plate as compared with the regular normal growth.

Immediately after addition of ———— bacteriophage. - - - After 24 hours' incubation of the tubes. o o o After 48 hours' incubation of the tubes. — . — . After 14 and 25 days' incubation.

irregular colonies, as compared with the regular colonies, on the plates inoculated from young bouillon cultures of B. typhosus when a bacteriophage is added, we were led to believe that the susceptibility of the bacteria is greater at the hours of incubation which produce the most irregular colonies, or even sterile plates. The filtrate from tubes showing the maximum amount of irregularity on plates were much more virulent than the filtrates from the tubes showing more regular growth.

This has led us to record our results in graphic form. The abscissa is the time in hours of the age of the bouillon culture of the bacteria at the time the bacteriophage was added. The ordinate, from above downwards, represents the degree of irregularity of all the colonies present expressed in percentages, from regular colonies (i.e., no trace of bacteriolytic activity), 25 per cent, 50 per cent, 75 per cent, 100 per cent and sterile. We do not wish to convey the idea that a culture will pass through all of these stages

gradually in an orderly manner. Sudden sterility has been observed, also a sudden return from a sterile plate to one showing a good growth of regular normal colonies. We have been using this chart method in recording our results in this laboratory for the past two years, and we wish only to use this method of expression in order to save space and show clearly our results. (Fig. 1.)

It will be observed that after 24 hours' growth the 3 hour old culture of B. typhosus (Olsen) was rendered sterile by the addition of the bacteriophage.

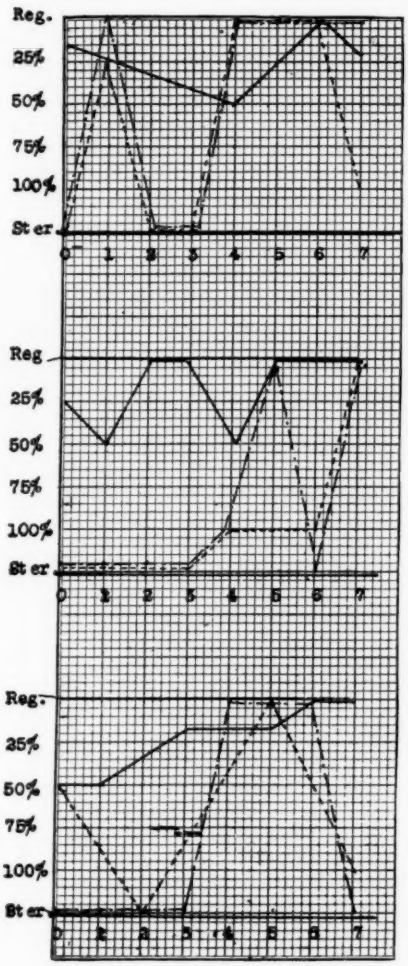

Fig. 2.

Fig. 3.

Fig. 4.

The 8 and 10 hour old cultures after 24 and 48 hours' incubation show decreasing amount of bacteriophage activity. The 14 and 25 day old cultures show that an equilibrium has been reached, only the 3 hour old culture tube shows any change and this remains sterile.

The descent of the curves from all of the ages of the cultures after the seventh or eighth hour, up to the time equilibrium is reached, from a regular colony growth to varying degrees of irregularity and even sterility, is striking. There is usually a heavy growth, especially in the immediate plating, with clear areas or "appearances." We think that d'Herelle and others,

who have thought these "appearances" to be "bacteriophage colonies" and have by an enumeration of such appearance attempted to determine quantitatively the bacteriophage present, have been using this very variable factor as a constant.

The same technic was followed in this experiment as was described in the preceding one. Four sets of tubes were used, one set was inoculated with an 18 hour bouillon culture; one from a suspension in bouillon of colonies from an agar culture 24 hours old, one from a 48 hour old agar colony supension, and the remaining set from a 72 hour old agar colony suspension in bouillon. The bacterial suspensions were made in bouillon to compare in density with those of the 18 hour bouillon culture; Gate's method in determining density of the suspensions was used.[9] (Figs. 2, 3 and 4.)

We give the results of this experiment to show that the age and also the medium upon which the susceptible microorganism has been grown, all must be taken into consideration in dealing with a "bacteriophage substance." This is particularly true when one uses such methods as d'Herelle and others have used for determining quantitatively the amount of bacteriophage present. Without a knowledge of the source of the culture and its history preceding the time it is put in contact with the bacteriophage, any quantitative estimation based upon the number of "appearances" or clear areas on the agar plate are valueless. We do not think that enough is known about this phenomena to enable one to attempt quantitatively to determine the amount of "bacteriophage substance" present in any given medium.

SUMMARY

"Bacteriophage Substance" or the bacteriolytic agent is adsorbed into the agar upon which the lycogenic colonies are grown.

A method is given to obtain a filtrate containing an active bacteriolytic substance that is free from proteins of the bouillon or nutrient culture medium. The addition of alcohol does not cause any precipitate in these filtrates.

A bacteriolytic substance added to a 3 hour old culture of susceptible bacilli of the typhoid-dysentery group causes a marked increase in the lytic power of this substance, not only for the homologous strain, but also for all other members of this group. The bacteriolytic activity is not only influenced by the age of the bouillon culture to which it is added, but is also dependent upon the age of the culture used to inoculate the bouillon medium and as to whether it was grown on a solid or fluid medium before inoculation.

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THE EFFECT OF A NORMAL MEAL UPON THE BLOOD SUGAR LEVEL IN HEALTH AND IN CERTAIN CONDITIONS OF DISEASE*

A Simple Food Tolerance Test

By I. C. Brill, A.B., M.D., Portland, Oregon

THIS study was undertaken with a view to developing a simple method for the determination of abnormal glycemic reactions. It is intended as a substitute for the glucose tolerance test now in general use in clinical and metabolic laboratories. This latter test is the result of the work of many authors, notably Bang, Hopkins, Hamman and Hirchman, and Janney and Isaakson. The value

CHART I NORMALS

CASE	STUDENT	BLOOD SUGAR FASTING	BLOOD SUGAR 1½ Hrs. AFTER BREAKFAST	CURVE
1.	Mr. L	0.110	0.110	110 — 110
2.	Miss S	0.090	0.090	90 — 90
3.	Mr. E	0.095	0.086	95 — 86
4.	Mr. H	0.074	0.079	74 — 79
5.	Miss B	0.069	0.081	69 — 81
6.	Miss C	0.093	0.073	93 — 73
7.	Miss S	0.096	0.096	96 — 96
8.	Mr. F	0.088	0.080	88 — 80
9.	Mr. I	0.105	0.110	105 — 110
10.	Mr. C	0.110	0.091	110 — 91
11.	Mr. A	0.095	0.080	95 — 80
12.	Mr. B	0.074	0.067	74 — 67
13.	Mr. C	0.074	0.080	74 — 80

Chart I.—The results of the glycemic reactions in thirteen normal individuals—all healthy students of the University of Oregon Medical School. It will be noted that the lines obtained are almost horizontal. The highest rise is 12 mg., while some present even a slight fall.

of the procedure as an aid in the study of various metabolic disorders is too well recognized to require further comment at this time. Some three years ago, while studying a number of cases of suspected and of some early known diabetes, it occurred to me that the use of a comparatively large quantity of glucose (which is necessary in the performance of the glucose tolerance test) is not entirely free from objections. First, the mixture is nauseous and distasteful to the patient; second, there may be some element of danger (theo-

*From the University of Oregon Medical School, Portland, Oregon.
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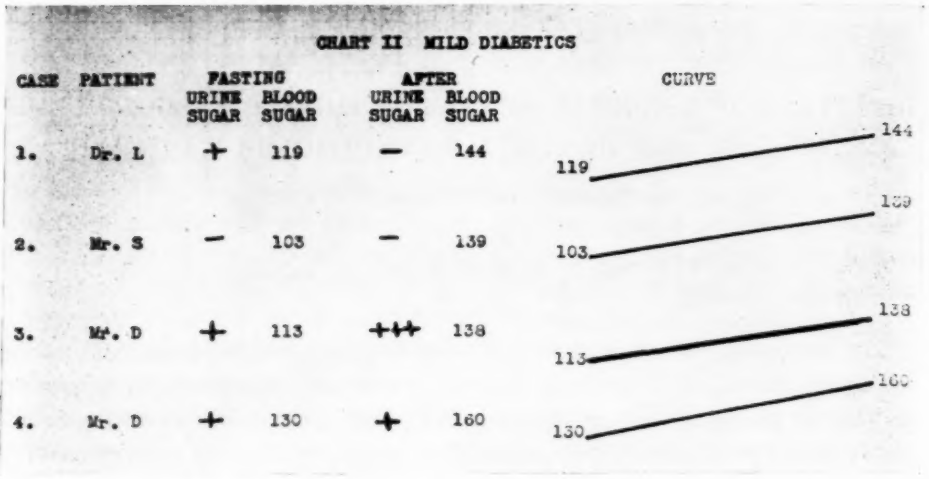

Chart II.—The result in known mild diabetes. Note all show a definite rise of from 25 to 36 mg. The fasting level is also apt to be higher than in the normal, although in mild cases it may not be so. In this group the fasting level was within normal limits in three of the four cases.

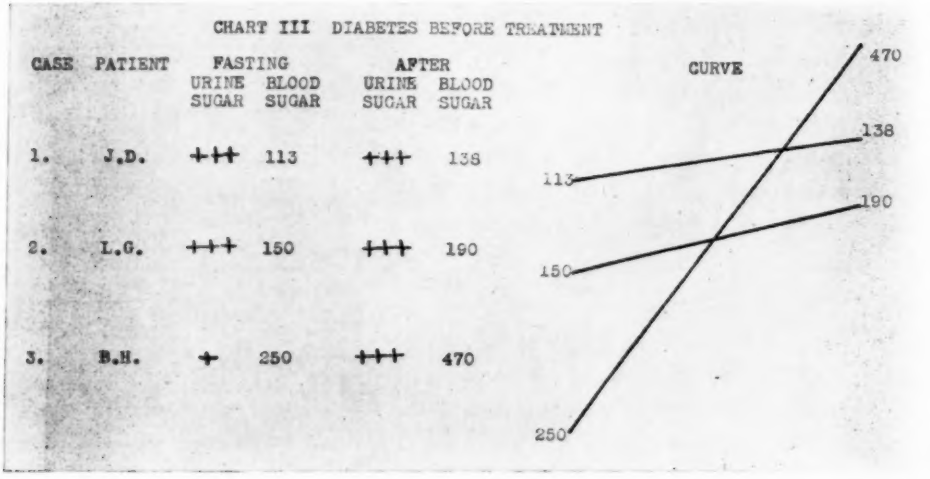

Chart III.—Results in three cases of diabetes, mild, moderately severe and severe, respectively.

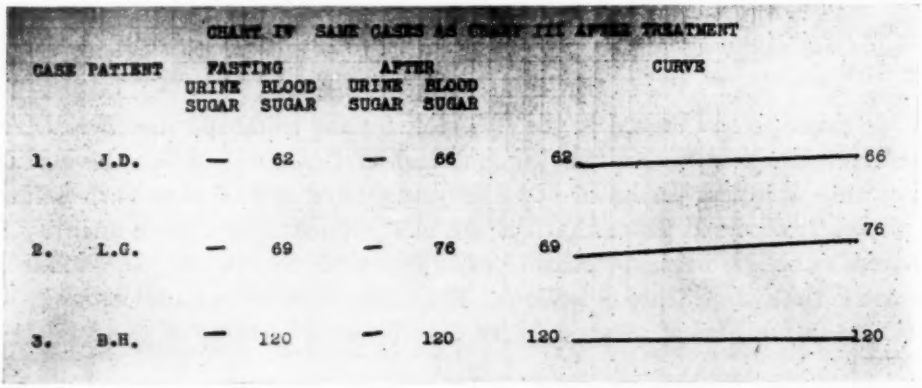
CHART IV SAME CASES AS CHART III AFTER TREATMENT
CASE PATIENT FASTING URINE SUGAR BLOOD SUGAR AFTER URINE SUGAR BLOOD SUGAR CURVE
1. J.D. — 62 — 66
2. L.G. — 69 — 76
3. B.H. — 120 — 120
62
66
69
76
120
120

Chart IV.—Results in same three cases as shown in Chart III after treatment.

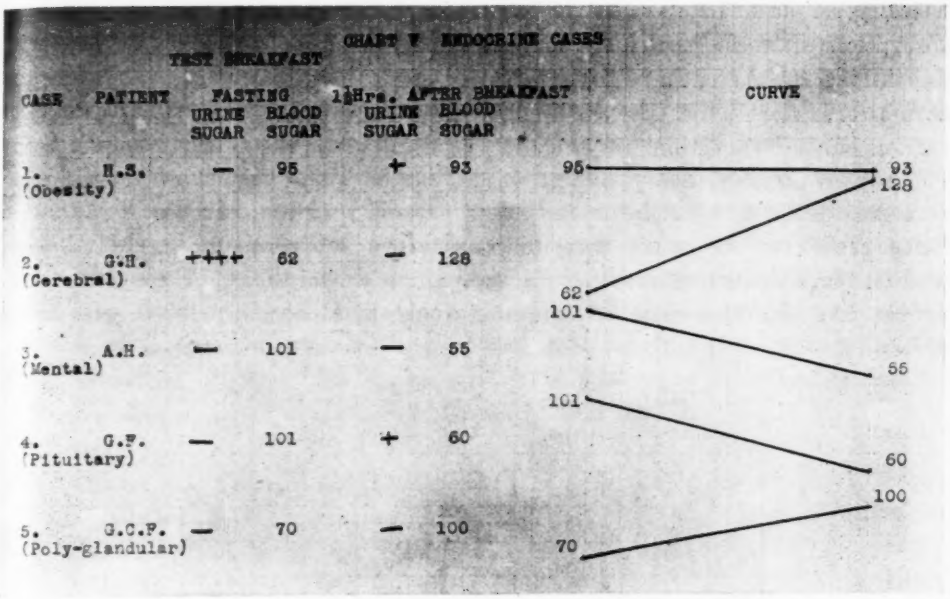
CHART V ENDOCRINE CASES
TEST BREAKFAST
CASE PATIENT FASTING URINE SUGAR BLOOD SUGAR 1½Hrs. AFTER BREAKFAST URINE SUGAR BLOOD SUGAR CURVE
1. H.S. (Obesity) — 95 + 93
2. G.H. (Cerebral) ++++ 62 — 128
3. A.H. (Mental) — 101 — 55
4. G.F. (Pituitary) — 101 + 60
5. G.C.F. (Poly-glandular) — 70 — 100
95 93
62 128
101 55
101 60
70 100

Chart V.—Results of the study of five endocrine cases. One of these cases showed nothing abnormal except for obesity. It will be noted that her glycemic reaction was practically normal. The other four cases all showed definite endocrine disorders and they all gave characteristic irregular glycemic reactions.

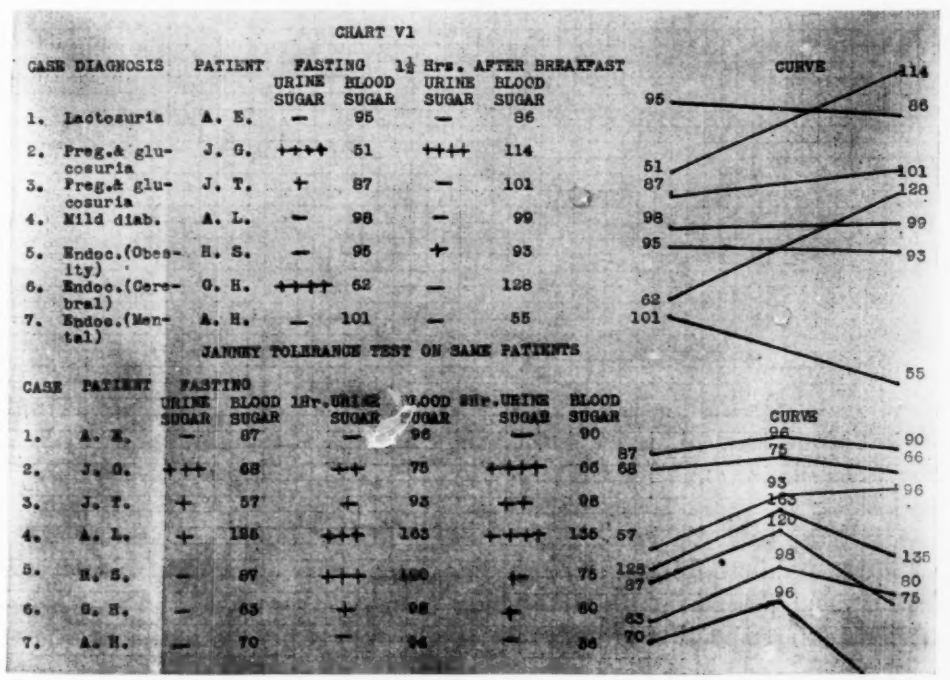
CHART VI
CASE DIAGNOSIS PATIENT FASTING URINE SUGAR BLOOD SUGAR 1½ Hrs. AFTER BREAKFAST URINE SUGAR BLOOD SUGAR CURVE
1. Lactosuria A. E. — 95 — 86
2. Preg.& glucosuria J. G. ++++ 51 ++++ 114
3. Preg.& glucosuria J. T. + 87 — 101
4. Mild diab. A. L. — 98 — 99
5. Endoc.(Obesity) H. S. — 95 + 93
6. Endoc.(Cerebral) G. H. ++++ 62 — 128
7. Endoc.(Mental) A. H. — 101 — 55
JANNEY TOLERANCE TEST ON SAME PATIENTS
CASE PATIENT FASTING URINE SUGAR BLOOD SUGAR 1Hr. URINE SUGAR BLOOD SUGAR 2Hr. URINE SUGAR BLOOD SUGAR CURVE
1. A. E. — 87 — 96 — 90
2. J. G. +++ 68 ++ 75 ++++ 66
3. J. T. + 57 + 93 ++ 96
4. A. L. + 125 +++ 163 ++++ 135
5. H. S. — 87 +++ 120 + 75
6. G. H. — 63 + 98 + 80
7. A. H. — 70 — 96 — 86
95 86
51 114
87 101
98 99
95 93
62 128
101 55
87 90
68 66
57 96
125 135
87 75
63 80
70 96

Chart VI.—The results of seven cases in which this test was used as an aid in establishing the diagnosis. In all these cases the findings were further checked up with the glucose tolerance test and as may be seen from the chart, the results were quite parallel. Two of these cases deserve special notice. They were cases of pregnancy with well marked glucosuria. (Cases 2 and 3). The question of terminating the pregnancy came up in each instance. Upon the basis of the findings by our test, I recommended to allow the pregnancy to continue. Only moderate restriction in carbohydrates was advised. Both cases were carried to a successful delivery without the slightest mishap. Both the mother and the child in each instance are living and well today.

retically, at least) in overloading a weakened tolerance by rapid and sudden absorption of a comparatively large quantity of pure glucose. These considerations led me to make a comparative study of the effects of an average normal breakfast upon the blood sugar content in normal individuals and in various chronic conditions in which the food tolerance is known to be affected. The results obtained are presented in the accompanying charts. The studies here recorded were all done in the latter part of 1919 and early part of 1920. Since these records were compiled, numerous other cases were similarly studied with identical results. However, in order to avoid repetition of figures and for the sake of clearness, only the original charts are here presented.

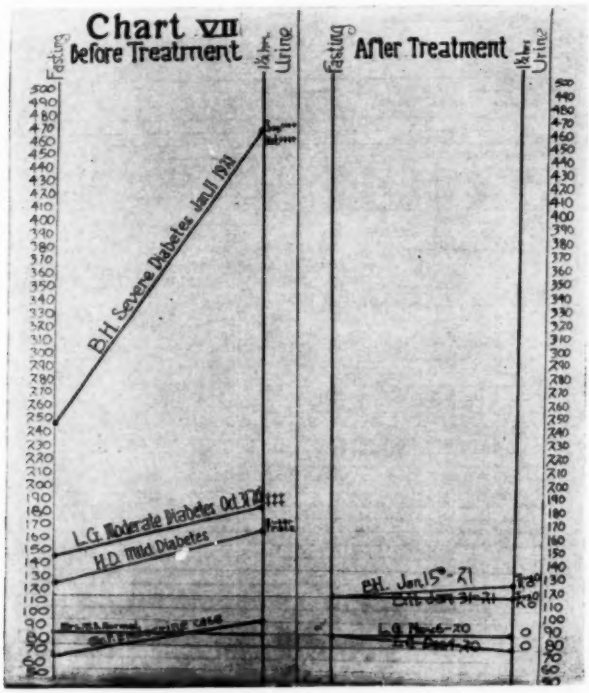

Chart VII.—Summary of the previous charts. On the left side of the mid-line curves are shown of the cases before they were treated. The dates of the estimations and the diagnoses are indicated. On the right side of the mid-line, the same cases with the dates of the subsequent estimations are shown. The same scale is used in order to show the marked contrast in the curve in different conditions, and in the same conditions before and after treatment.

In making this comparative study, the following factors were considered:

First, *the time*. It was shown by Mosenthall and others that in diabetes the maximal percentage of blood sugar occurs between one and two hours after breakfast and that after luncheon and evening meals the rise is not so constant and the sugar may, in fact, even fall. On the other hand, in normal individuals, I have found the blood sugar to be most nearly constant after the morning meal, while, after luncheon, it is apt to rise higher than normal. Breakfast time, therefore, will afford the greatest contrast between the normal and the abnormal conditions.

Second, *the meal.* In order that the test may be a true food tolerance test all the food elements should be represented in the test meal, and it therefore included the usual breakfast foods, namely: bread, butter, cereals, eggs, sugar, coffee and cream, amounting to approximately 100 grams of carbohydrates, 26 grams of protein, 27 grams of fats and 760 calories.

Third, *the curve.* Two estimations only were done, fasting and an hour and a half after breakfast. These two estimations form a line which may be horizontal, rising or falling. Normally an hour and a half after breakfast the blood sugar is within ten milligrams of the fasting level and the two estimations furnish almost a horizontal line. In diabetes, however, a greater difference is found, the second level being considerably higher than the first and, therefore, a rising line is obtained. The difference may best be learned by an examination of Charts 1 and 2.

CONCLUSION

1. A comparatively simple method is suggested to serve as a true tolerance test.

2. In all the cases tried so far, it corresponds very closely in its results with the Janney glucose tolerance test.

3. It is preferable to the glucose tolerance test because (a) it is more simple, (b) it is more agreeable to the patient, (c) and it avoids the danger of overtaxing a weakened function by a large quantity of pure glucose.

I wish to express my thanks to Prof. Howard D. Haskins from the department of Biochemistry of the University of Oregon Medical School for assisting me in many of the blood sugar estimations, the results of which form the basis of this study.

THE DIFFERENTIAL LEUCOCYTE COUNT IN CHRONIC PERIAPICAL DENTAL INFECTION.*

By Russell L. Haden, M.D., Kansas City, Mo.

AT the present time the radiograph is necessarily the main dependence in the diagnosis of chronic periapical dental infection. Clinicians and dentists agree that patients suffering from systemic disease of local origin should have removed all teeth which may be a source of infection. There is a great difference of opinion, however, as to what should be done with pulpless teeth which are negative in the radiograph, and even with teeth which show evidence of infection in the absence of systemic disease. The problem is of great concern to both the physician and dentist. It is of special interest to the dentist since the patient presenting himself to him is so often not suffering from systemic disease, while the reverse is true of a patient coming to the physician.

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From the Deaner Institute.

The criteria of systemic disease are relatively crude and the physical signs relatively gross. Great damage may be done to body tissues before the changes are serious enough to be recognized by the usual clinical methods. It is apparent that foci which are causing general damage should be removed early as a preventive measure. Some diagnostic sign which would enable us to determine whether absorption is taking place from infected areas in the absence of systemic disease would be of the greatest value in indicating the proper procedure to follow with pulpless teeth. Such teeth may be negative in the radiograph and yet be infected, since there must be many bacteria present before sufficient bone destruction takes place to show in the x-ray. On the other hand teeth around which there is evident bone destruction may have become sterile and thus be harmless from a systemic standpoint.

Attempts have been made to determine toxin absorption from infected foci by the complement-fixation method. Mixed cultures or organisms isolated from periapical infection are used as an antigen. Such procedures have yielded little information of value. A study of leucocytes of the blood would seem to be a more promising method of approach, since the white corpuscles may respond quite rapidly when bacteria or products of bacterial activity are absorbed by the blood.

Hecker states that he has observed tinctorial changes in the leucocytes in the presence of dental infection.[1] Logan found a leucocytosis in 47 out of 52 cases of periapical infection.[2] Goadby thinks that there is constantly an increase in mononuclears in pyorrhea and systemic disease.[3] Daland concludes that "small cell lymphocytosis with a corresponding decrease in the polymorphonuclear cells is an important diagnostic sign of periapical dental infection, the value of which is increased when leucopenia exists."[4] This observer considers that lymphocytosis (38 per cent or more of lymphocytes) indicates that toxins or streptococci or both are entering the blood stream. He states that he found a lymphocytosis only twice in 100 cases of chronic disease where no focal infection existed. Toren claims to have found a certain type of leucocyte in the blood indicative of infection about the teeth.[5] This cell, he thinks, originates in the lymph glands draining the infected area.

To determine whether we could utilize the findings described by others as a diagnostic measure in chronic periapical dental infection, I have made a differential leucocyte count on blood films from 200 patients. The films have in every instance been made on cover glasses, a procedure which allows the drop of blood to spread spontaneously and evenly. It is very doubtful whether it is possible to get a true differential blood count where films are made on slides. No films have been used unless there was a uniform spread of the cells with the red cells one cell thick over an area covering at least 8 low power fields of the microscope. On each film 500 cells have been counted, hence the study includes a count of 100,000 cells. All the counts have been done by the author.

The films have been made on 7/8" cover slips and stained with Wright's stain as follows: eight drops of stain are run on the film and left for one minute, eight drops of a phosphate buffer solution of P_H 6.4 are then added and left

for four minutes. The film is washed with distilled water, dried and mounted film side down in balsam. We have found that the addition of the buffer solution instead of distilled water to the stain gives remarkably uniform preparations and differentiates very sharply the various types of white cells. The granules of the granular cells are brought out especially brilliantly in films prepared in this manner.

In such a study it is apparent that the basis of classification of the leucocytes should be plainly stated. This is not clear in many published reports. Daland, for instance,[4] appears to have divided the cells only into lymphocytes and polymorphonuclears. Toren in claiming that there is a peculiar type of cell in infection about the mouth evidently uses a different classification from other hematologists, since he finds in normal individuals only 1-2 per cent large mononuclears while others agree on 6-10 per cent.

From the standpoint of origin there are three different types of white cells in the blood (1) polymorphonuclears coming from the bone marrow, (2) lymphocytes, large and small, arising in the lymph glands and (3) true monocytes, which include both the large mononuclears and transitionals. The origin of the latter is in dispute, but the consensus of opinion is that they are of endotheloid origin. The identification of the polymorphonuclears is simple. The same may be said of the small lymphocytes. There is a great deal of confusion, however, concerning the relation and differentiation of large lymphocytes and large mononuclear cells. In films stained by the technic described above, the mononuclear cells are sharply differentiated. The large lymphocytes have dark blue nuclei and the protoplasm is clear or diffusely blue. The protoplasm may contain azurophile granules, which are a very dark purple color, are usually few in number and show a great variation in size. The true large mononuclears, on the other hand, have relatively larger nuclei which may be round, indented, or saddle-bag shaped and usually take a lighter stain than the nucleus of the lymphocytes. The protoplasm has a ground glass appearance due to the uniformly distributed light purplish blue granules which are very fine and of uniform size. The granular appearance has been ascribed as due to nodosities in the reticulum of the protoplasm, but seems more likely due to the presence of true granules.

The classification we have used has the added value that it can be compared with the very complete study of the normal differential leucocyte counts made by Miller.[6] Miller includes the large lymphocytes with the large mononuclears and makes a separate classification of the transitionals. The lymphocyte group is thus smaller and the large mononuclear group larger than would be when the cells are classified on the basis of origin.

Patients have been grouped as follows:

Group I—Includes 134 patients who showed evidence of alveolar abscess in the dental radiograph. Since we have proved by culture that many such abscesses are sterile it does not necessarily follow that all the individuals of this group really have periapical infection.

Group II—Includes 66 patients who showed no definite evidence of infection in a radiograph of the teeth. 28 of the 66 patients in this group had

one or more pulpless teeth. Many pulpless teeth which show no radiographic evidence of infection are really infected, so here again the grouping is rather artificial.

Group III—Comprised 47 patients who showed, on careful physical examination, definite evidence of systemic disease of focal origin. The diseases in-

TABLE I

		GROUP	I	II	III	IV	V
	TOTAL NUMBER OF COUNTS		134	66	47	53	25
		Average	8311	7800	8783	7714	8838
			%	%	%	%	%
Total White Blood Cell Count	Distribution of Counts	Over 10,000	11.8	7.5	27.6	9.5	24.0
		8000-10,000	42.2	33.3	36.2	30.3	44.0
		6000-8000	38.5	52.8	29.8	45.5	20.0
		4000-6000	7.5	6.1	6.4	15.2	12.0
		Average	64.4%	62.2%	63.9%	64.6%	66.2%
		Over 80%	2.2	1.5	2.5	0.	0.
Polymorpho-nuclear Neutrophiles	Distribution of Counts	70-80%	23.9	4.2	12.7	40.0	16.0
		60-70%	47.9	34.8	63.8	32.1	60.0
		50-60%	23.9	36.4	19.2	22.7	24.0
		40-50%	2.2	3.0	2.0	5.2	0.0
		Average	1.7%	2.0%	2.3%	1.6%	2.5%
			%	%	%	%	%
		Over 5%	3.0	3.0	6.3	0.	8.0
Polymorpho-nuclear Eosinophiles	Distribution of Counts	4-5%	3.0	3.0	6.3	0.	8.0
		3-4%	8.2	6.1	15.0	9.4	8.0
		2-3%	20.2	30.3	27.7	24.5	36.0
		1-2%	44.8	31.8	25.5	41.5	16.0
		Under 1%	20.8	25.7	19.2	24.5	24.0
		Average	0.5%	0.4%	0.4%	0.4%	0.5%
			%	%	%	%	%
Polymorpho-nuclear Basophiles	Distribution of Counts	Over 1%	3.8	6.0	10.6	16.9	16.0
		0.4-1%	73.1	72.7	59.5	37.7	28.0
		Under 0.4%	23.1	21.3	29.9	45.5	56.0
		Average	27.1%	28.5%	26.0%	27.5%	26.6%
			%	%	%	%	%
		Over 30%	29.9	45.5	23.4	35.9	28.0
Lymphocytes (large and small)	Distribution of Counts	25-30%	31.3	24.2	25.5	17.0	40.0
		20-25%	21.6	18.2	38.3	24.5	16.0
		10-20%	16.4	12.1	12.8	22.6	16.0
		Under 10%	0.8	0.	0.	0.	0.
		Average	6.2%	6.5%	6.5%	5.9%	6.3%
			%	%	%	%	%
		Over 10%	4.5	6.0	10.6	7.5	8.0
Large Mononuclears and Transitionals (Monocytes)	Distribution of Counts	8-10%	5.9	12.1	23.4	9.4	24.0
		6-8%	14.2	21.2	23.4	15.1	16.0
		4-6%	67.2	53.0	19.2	54.7	24.0
		Under 4%	8.2	7.6	23.4	13.3	28.0

cluded are arthritis, neuritis, iritis, pyelonephritis, duodenal ulcer, encephalitis and endocarditis. All patients had pulpless teeth and 36 had periapical abscesses.

Group IV—Includes 53 patients who were thoroughly examined and found to have no evidence of systemic disease; 29 of this group had pulpless teeth and 17 had periapical abscesses.

Group V—Comprises 23 patients, all of which were proved by culture to have infected teeth. In every case rabbits were injected and the lesion from which the patient suffered reproduced in the animal. There is every reason to believe that the dental infection was the source of the systemic disease. If the differential blood count shows anything typical in focal infection it should certainly be evident in this group.

The results of the differential counts are shown in Table I. The average white cell count in Group I is 8311 per c.mm., Group II, 7800; Group III, 8763; Group IV, 7714, and Group V, 8838. The differential count is remarkably alike in the five groups. The average polymorphonuclear neutrophile is 62.2 per cent in Group II and 66.2 per cent in Group V. These are the two groups which one would expect to show the greatest difference. The eosinophiles show much the same variation, being highest in Group V. The basophiles show no change. The lymphocytes show practically the same average throughout the five groups. The average is 26.6 per cent in those patients showing undoubted evidence of disease of focal origin (Group V) and 28.5 per cent in those showing no evidence of infection in the radiograph (Group II). The large mononuclear cells show equally little variation.

DISCUSSION

The results in this series of counts indicate that the patient with alveolar abscesses will have a slightly higher total white count than one who shows no radiographic evidence of infection. If systemic disease of focal origin is present the difference is somewhat more marked. This difference is not great enough, however, to be of any definite value in diagnosis and decision as to treatment.

A study of the differential counts shows that the increase in white cells observed is due to a general increase in all types of cells, although there is a slight relative increase in the polymorphonuclear cells. There is no relative increase in the lymphocytes and large mononuclear cells. We have found no evidence of any type of cell which is peculiar to focal infection.

There is no sufficiently clear-cut increase in any one type of cell to make the differential leucocyte count of definite value in the diagnosis of absorption from areas of infection. In fact, the study has demonstrated to us that the differential leucocyte count is of little value in our problem.

SUMMARY

The results of the study of the white cell count and differential count in 200 patients is presented; 100,000 white cells have been counted.

Patients with periapical abscesses show a slightly higher total white count than those having no abscesses. The difference is somewhat more marked in those patients suffering from systemic disease of focal origin.

The increase is, for the most part, in the polymorphonuclears, although all types show some increase.

Patients suffering from chronic periapical dental infection do not show typically a lymphocytosis.

We have found no evidence of an unusual type of white cell or tinctorial reaction in chronic dental infection.

The differential count is of little practical value in determining whether a patient is absorbing toxins or bacteria from possible foci of infection about pulpless teeth.

Group I. Patients showing in radiograph definite evidence of one or more infected teeth.

Group II. Patients showing in radiograph no evidence of infected teeth.

Group III. Patients with infected teeth and systemic disease of focal origin.

Group IV. Patients with no evidence of systemic disease of focal origin (17 showed definite areas of rarefaction in dental radiograph, 28 had no pulpless teeth, and 18 had pulpless teeth showing no evidence of infection).

Group V. Patients with systemic disease of focal origin which was reproduced in rabbits by bacteria isolated from periapical foci.

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CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

By A. Levinson, M.D., Chicago, Ill.

(*Continued from page 678.*)

Stool

THE first requirement in the interpretation of stool of infants is to examine it immediately after or only a short time after it has been dejected from the infant. Changes in color and consistency take place in stools on standing. Often a stool that is light yellow or deep yellow in color to start with, will take on a greenish or real green color on standing one or more hours. A stool that has been watery to start with often dries up on standing, leaving no residue or leaving only traces of stain on the diaper. One must also take into consideration the type of food ingested by the infant, especially the sugar, as the food influences the color, size, and consistency.

The next condition in examination of infant's stool is that no cathartic or laxative should have been given the baby on that day, for every cathartic changes the appearance of the stool. Castor oil, for instance, produces mucus in the stool even in a normal baby, and calomel gives a green color, whether or not there is intestinal disturbance to begin with. What is true of physics is also true of other medications. Charcoal, for instance, shows black particles in the stool and argyrol colors the stool brown.

EXAMINATION.—The most important points in the examination of infant's stools are: number, size, consistency, color, odor, curds, reaction, fat, mucus, pus, blood, bacteria, and parasites. Size, color, and curds are determined by the naked eye. Reaction is determined by litmus paper, or by titration (easiest by litmus paper); fat is determined by sudan III or galactometer. Blood is determined macroscopically, microscopically, or by the guaiac test; pus is determined microscopically. Bacteria are determined both on slide and on culture media, special tubes and special media being used for culturing stools (see section on smears and cultures). Parasites are determined by the naked eye or by the microscope, parasitic ova are determined microscopically.

The number of stools in healthy infants and children varies between 1 to 3. Some infants and children will, however, have only one bowel movement in 36 hours with untoward effects on their health. The food ingested also influences the number of stools. Boiled milk tends to constipate, vegetables tend to increase the number of stools.

The size of the stools depends on the age of the child; the number of stools, the character of the food, and the condition of the gastrointestinal tract. Infants' stools are necessarily smaller, as infants' food contains relatively less residue. When the diet is increased by cereals and vegetables, the stools increase in size because of the increased residue. In cases of starvation and partial intestinal obstruction, such as pyloric stenosis, chronic intussusception, and malformation of the rectum, the stools are small. In dilatation of the colon, Hirschsprung's disease, the stools are infrequent and very large—constipated stools tend to be large. In most cases of diarrhea the stools are small and numerous. In tenesmus due to any cause the stools are frequent and small.

The consistency of the stools varies with the diet and condition of the digestive tract—the thin fluid stool seen in diarrhea, often on relatively high carbohydrate feeding; the normal semiformed or soft stool seen in thriving infants and children; the firm formed and even hard stool in constipated children.

Color depends a great deal on the type of food ingested and on the time the stool has been standing before examination. Breast feeding gives a deep yellow stool. Cow's milk alone, or with cane sugar, also gives a yellow color. The addition of dextri-maltose to the milk changes the stool to a light brown, Mellin's Food changes it to a dark brown, and honey changes it to a light yellow.

One way of testing whether the stool has been green to begin with, or has become so on standing, is to examine the inner part of the stool. If the green color is due to oxidation, only the outside will be green, the rest being yellow. If the green is formed in the intestine, this color will be seen all through the stool. Green stool (the color being present in fresh stool) occurs in nearly all cases of diarrhea.

Normal infant's stool has no odor at all or it smells "sour." An offensive odor indicates disturbed digestion.

Large bean-shaped curds are often present in the stool when an infant

is fed on raw milk. As soon as the milk is boiled or sodium citrate is added to the milk, the large curds disappear. Small curds varying in size from a pinhead to a linseed are present even in normal stools. When present in large numbers they may be indicative of disturbed fat digestion.

The reaction of a normal stool is neutral, faintly acid, or faintly alkaline. This may be ascertained by the use of litmus paper. If the stool is formed a suspension may be made with a small portion of stool and a few c.c. of distilled water. Diarrheal stools tend to be acid because of the presence of fermentation products. A high carbohydrate intake favors fermentation. When large amounts of fat are ingested, the resulting fatty acids tend toward acid stools. When high protein feedings are given there is putrefaction with resulting alkaline stools.

Fat in stools may be seen as neutral fat, fat soaps, and fatty acids. To examine for fat, a small amount of stool is smeared on a glass slide and a drop of sudan III stain added. This colors neutral fat bright red. Soaps do not stain. Fatty acids stain orange red. When a weak solution of carbol-fuchsin is used instead of sudan III the soaps stain a faint rose, the neutral fats do not stain, and the fatty acids are red. The crystals of fatty acids may be seen by adding a drop of acetic acid to the smear, and heating for a minute or so, and then allowing it to cool.

The presence of starch may be demonstrated by the addition of iodine which gives the starch granules the usual blue-black color.

Mucus may be seen as white, shiny, tenacious masses of varying amounts. Pus may be observed both macroscopically and microscopically.

Blood in the stool may impart a dark or tar color to the stool, as is the case when the hemorrhage is high up in the intestinal tract. Fresh blood, from rectal fissure, polyps or ulcerations low in the intestine, appears bright red and is not thoroughly mixed with the stool. Sometimes blood is present in very small amounts and can be determined only microscopically or by chemical tests.

The Guaic Test for Blood.—A water suspension of stool is prepared and one-third volume of glacial acetic acid is added and thoroughly mixed. If blood is present the coloring matter is thus converted to acid hematin. The mixure is now filtered and extracted with two or three volumes of ether. Fresh tincture of guaiac is prepared by dissolving a knife point of powdered guaiac in 5 c.c. of alcohol. About 2 c.c. of the ether extract is treated with 10 drops of the tincture of guaiac and 25 drops of hydrogen peroxide are added. After thorough mixing the presence of blood is evidenced by a blue color which fades after standing.

Parasites.—Worms, especially round worms and tape worms, can be detected with the naked eye. Pin worms can also be found if looked for carefully. The mother may best do this when the child goes to bed. In looking for tape worm segments, the search can be facilitated by tying a large strip of gauze over the opening of the bed pan in which the stool is to be obtained. This is placed in the sink under running cold water for an hour or two and the parasites are looked for in the residue. In looking for ova, a substantial

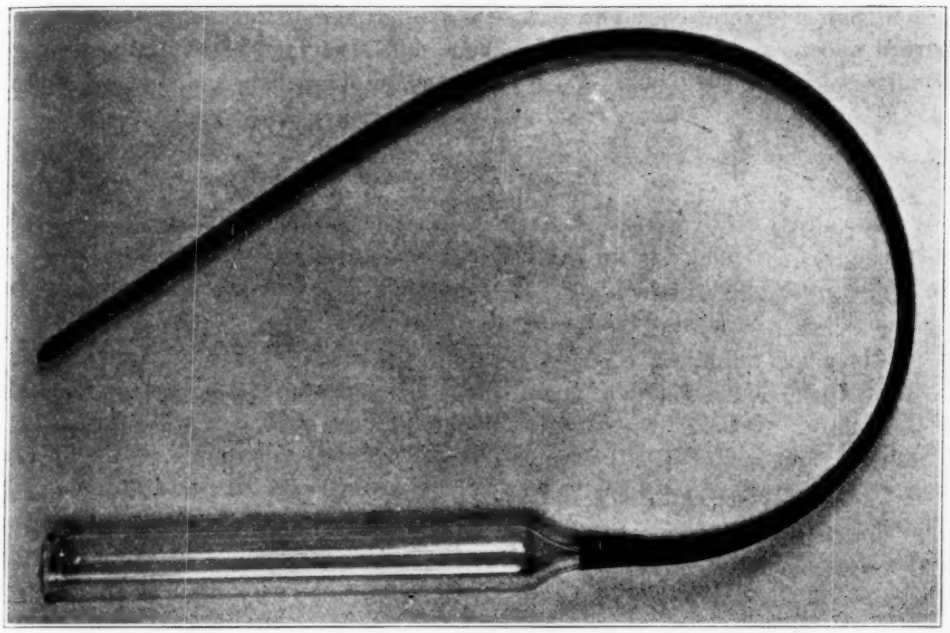

Fig. 30.—Barrel of glass syringe and catheter used advantageously for gavage.

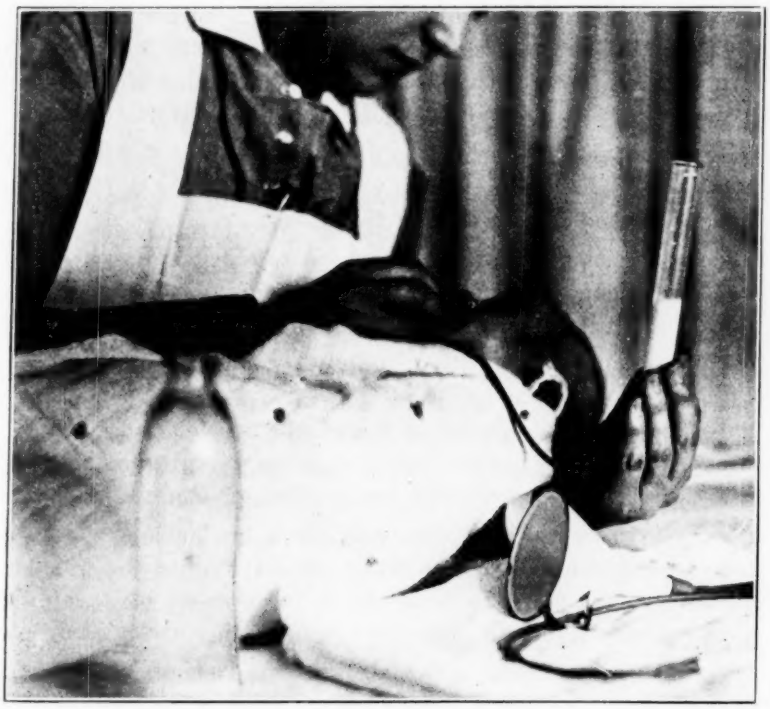

Fig. 31.—Gavage.

amount of the stool is rubbed up thoroughly with several volumes of a saturated chloride solution. The parasites rise to the surface, are scraped off with a spoon, and set aside for one hour. The surface of the fluid is now skimmed with a wire loop ½ to ⅔ inches in diameter, and several loops placed on the slide. This is examined under low power.

Lavage and Gavage

Indications.—Lavage is indicated in cases of severe vomiting due to food disturbances, especially when the vomitus contains large quantities of mucus. It is also indicated in poisoning. It is employed occasionally in older infants and children who refuse food because of neurosis.

Gavage is indicated when the child refuses food; when he is too weak to take food; when there is a deformity of the mouth, such as in marked cleft palate; in cases of stricture of the esophagus; in profuse persistent vomiting; and in postdiphtheric paralysis.

Technic.—The child is wrapped in a sheet and held firmly on the nurse's lap, or laid down flat on the table. The tongue is depressed with a tongue depressor, if the child has teeth, or with the index finger of the left hand if the child has no teeth. The catheter held between the thumb and index finger of the right hand is now passed quickly into the esophagus; the index finger of the left hand in the pharynx being used as a guide for the catheter.

The catheter should be soft, 12 to 14 French for infants, and 18 to 20 French for older children, especially if gavage is to be given, and especially if thick food, such as cereal, is to be introduced into the stomach. An idea of the length of the catheter to be inserted may be obtained by measuring the distance between the tip of the child's nose and the ensiform cartilage.

A funnel is attached to the catheter by means of an extra piece of rubber tubing, and a short glass tube used as a window. The glass part of a medicine dropper may be conveniently used for the latter.

For lavage, a 2 per cent sodium bicarbonate solution, 1 per cent to 2 per cent boric acid solution, or plain warm water is used. The solution is poured into the funnel and allowed to run back by lowering the funnel. The procedure is repeated several times until no more food or mucus comes back with the solution.

For gavage the food is introduced into the funnel and allowed to run into the stomach gradually. The barrel of a ten c.c. glass syringe attached directly to a catheter will answer the purpose for gavage. (Figs. 30 and 31.)

A rubber apron for the physician and nurse, and a large basin as a receptacle for the stomach contents in lavage will facilitate the procedure.

Nasal feeding may have to be resorted to in cases of paralysis of the pharynx, especially in acute anterior poliomyelitis and postdiphtheritic paralysis.

The technic of nasal feeding is the same as in the ordinary gavage, except that the catheter is introduced through the esophagus by way of the nostril, instead of by way of the pharynx.

ESTIMATION OF THE AMOUNT OF PANCREATIC ENZYMES IN DUODENAL FLUID BY A MODIFIED GAUTIER'S METHOD I*

By George F. Spencer, M.D., Philadelphia, Pa.

MUCH has recently been written concerning the estimation of the gastric secretions with a determination of the acidity or the alkalinity. The duodenum has been explored by means of the duodenal tube and samples of duodenal secretions have been procured and examined both chemically and microscopically[1] but apparently the duodenal secretions, with particular reference to the pancreatic enzymes, have been practically overlooked.

One or two methods for estimating enzymatic activity in duodenal contents have been published[2] but apparently no practical method for the busy clinician has been brought forth. For that reason we propose to give you a method[3] for the determination of the pancreatic enzymes in duodenal fluid which is simple enough for any clinician to use, accurate enough for practical purposes and one which requires the minimum of time and expense to perform.

Procedure: Modification of Gautier's method.

(A) Prepare three 50 c.c. Erlenmeyer flasks marked T, A and L (trypsin, amylase and lipase).

(B) To flask T. add 20 c.c. of a 5 per cent solution of gelatine (1).

(C) To flask A. add 20 c.c. of a 5 per cent solution of soluble starch (2).

(D) To flask L. add 5 c.c. 20 per cent emulsion of olive oil (3).

(E) To each flask add one or two drops of a 1 per cent alcoholic phenolphthalein solution. Then measure into each flask by means of an Ostwald pipette 1 c.c. of the duodenal fluid to be tested.

(F) To each flask add drop by drop, from a burette, decinormal sodium hydroxide until a light pink color is produced which remains on shaking. At times it will be necessary to add, in the same manner, decinormal hydrochloric acid instead of sodium hydroxide, for, at times, the reaction will be found to be already alkaline, that is alkaline to phenolphthalein. Incubate for one hour at 37° C., shaking the flasks every fifteen minutes.

(G) Upon removal from the incubator, at the end of exactly one hour, place flasks T. and L. in ice water and add to flask A. a few grains of sodium carbonate to stop digestion.

(H) Controls of boiled duodenal fluid, plus gelatine starch and oil treated as above stated must be incubated with the three tests and correction for their blanks be made.

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Corring—Borden—Keen Research Fellow of the Jefferson Medical College.

ESTIMATION OF THE TRYPTIC ACTIVITY. CALCULATION

Direct acidity. Titrate gelatine digestion mixture to neutrality, (first light pink) with 0.1 N. NaOH, take the burette reading, subtract the blank reading: The result is a measure of the direct acidity developed by the ferment activity of 1 c.c. of the duodenal fluid upon one gram of gelatine during one hour's digestion at 37° C.

Total Acidity. Add 5 c.c. of neutral formol alcohol solution (4), titrate again to neutrality. Take the burette reading, subtract the blank reading. The result plus the figure for the direct acidity, is a measure for the total acidity due to the amino acids derived from the action of one c.c. of the duodenal fluid upon one gram of gelatine.

ESTIMATION OF THE LYPOLITIC ACTIVITY. CALCULATION

Direct Acidity. Titrate oil emulsion digestion mixture to neutrality with 0.1 N. NaOH, take the burette reading, subtract the blank reading. The figure thus obtained is a measure of the direct acidity developed by the action of one c.c. of duodenal fluid acting upon one c.c. of olive oil.

Total Acidity. Add 10 c.c. of neutral alcohol-ether solution (5) titrate again to neutrality, take the burette reading, subtract the blank reading. The result is a measure of the acidity developed by the fatty acids derived from the action of 1 c.c. of duodenal fluid upon 1 c.c. of olive oil for one hour at 37° C.

ESTIMATION OF THE AMYLOLYTIC ACTIVITY. CALCULATION

(1) *Maltose.* Pour starch solution digestion mixture into a 25 c.c. burette, run slowly, then drop by drop, into 25 c.c. of Benedict's reagent to which has been added 10 to 15 gm. of sodium carbonate. Estimate the amount of maltose present by Benedict's Quantitative Method for dextrose (6). Take the burette reading and note the number of c.c. required to cause a complete disappearance of the blue color of Benedict's Reagent (5). This figure divided into 0.0747, (the amount in grams of maltose required to reduce 25 c.c. of Benedict's Reagent) gives the amount of maltose in 1 c.c. of digested starch solution. Multiply by 20 to obtain the amount of maltose formed from one gram of soluble starch (5 per cent solution used).

(2) *Weight of starch utilized.* Multiply the maltose figure by 0.9473. This is the factor derived from 324/342. (One gram of soluble starch, the amount present in 20 c.c. of starch solution, contains about 7.7 per cent of water making the starch used in the experiment equal to 0.923 gm. To estimate the true amount of starch utilized, multiply the above figure by 0.923.

TO PREPARE A FIVE PER CENT GELATINE SOLUTION

Weigh out 50 grams of the highest grade culture media gelatine and add it to a liter of distilled water in a large beaker over a Bunsen burner. The temperature of the water should be kept below 60° C. to prevent scorching. Stir continuously until the gelatine solution is homogeneous. Pour carefully into a clean, previously heated, bottle. (Heated to prevent crack-

ing of the bottle.) Add toluol to cover the gelatine solution with one quarter inch surface. Keep at warm room temperature.

TO PREPARE A FIVE PER CENT SOLUBLE STARCH SOLUTION. (2)

Weigh out 50 grams of soluble starch. Stir into a smooth paste in a mortar, after adding slowly 50 c.c. of cold distilled water. Heat to boiling 950 c.c. of distilled water, add, with continuous stirring, the starch paste until a homogeneous mixture results. Pour carefully into a clean previously heated bottle and add toluol to cover to the depth of one quarter of an inch. Keep at room temperature.

TO PREPARE A TWENTY PER CENT OLIVE OIL EMULSION. (3)

Measure out thirty grams of powdered acacia, 60 c.c. of distilled water and 120 c.c. of the best grade of olive oil, stir the oil and the acacia into a smooth paste in a mortar. Always stir in one direction. When a thick homogeneous paste results, add quickly and in one amount 60 c.c. of water and continue stirring until a milk white emulsion is formed. Pour into a large graduate, add distilled water, with repeated washing of the mortar, until 600 c.c. of the emulsion is made. Add 1 c.c. of formalin as a preservative. Keep in the ice chest.

TO PREPARE AN ALCOHOL-ETHER SOLUTION. (4)

Take equal parts of 95 per cent alcohol and 40 per cent formalin, and mix. Make neutral with concentrated NaOH, that is, after adding a drop or two of phenolphthalein. Titrate the quantity to be used in the day's experiment to neutrality.

TO PREPARE AN ALCOHOL-ETHER SOLUTION. (5)

Five parts of neutral 95 per cent alcohol and one part of acid-free ether are mixed fresh for each day's experiments. Ten c.c. of this mixture made neutral with decinormal NaOH are used in each test.

TO PREPARE BENEDICT'S SUGAR REAGENT. (6)

Copper sulphate. (Crystallized)	18.0 gm.
Sodium carbonate. (Crystallized)	200.0 gm.
(One-half the weight of the anhydrous salt may be used.)	
Potassium thiocyanate	125.0 gm.
Sodium or potassium citrate	200.0 gm.
Potassium ferrocyanide. (5 per cent solution)	5.0 c.c.
Distilled water. qs. a.d.	1000.0 c.c.

With the aid of heat dissolve the carbonate, citrate and thiocyanate in enough water to make about 800 c.c. of the mixture and filter if necessary. Dissolve the copper sulphate in about 100 c.c. of distilled water and pour the solution slowly into the other liquid with constant stirring. Add the ferrocyanide solution, cool and dilute to exactly one liter. Of the various constituents the copper salt only need be weighed with extreme exactness. Twenty-five c.c. of the reagent are reduced by fifty grams of glucose.

To the majority of our readers it may, at first, seem as though we had not fulfilled our promise, that is that the method is not practical for the busy clinician, in that it requires too much time to perform. The author

here wishes to state that the time required to perform the entire set of determinations, including the calculation, is no more than one and one-half hours.

The reagents as we have shown are to be found in nearly all clinical laboratories and do not, when properly prepared, deteriorate on standing.

The accuracy of the method is best shown by the fact that out of five hundred or more determinations, all run in duplicate, we have yet to check outside the normal limits of error.

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A STUDY OF THE YEASTS FOUND IN THE SPUTUM OF PATIENTS WITH ASTHMA AND CHRONIC BRONCHITIS*

EDWARD STEINFIELD, M.D., PHILADELPHIA, PA.

THE description of fungi found in cases of bronchomycosis in the tropics has been considerably amplified by the studies of Castellani.[1] Among these fungi were recorded those of the genera Monilia, Saccharomyces, Cryptococcus, Oidium, Aspergillus, Sporotrichum and others. The elaborate classification of the Monilia, particularly by biochemical reactions has been especially applicable to the observations outlined in this paper. The occurrence of large amounts of yeast spores as well as vegetative forms in the sputum of patients living in Philadelphia and suffering from chronic bronchitis or asthma, suggested a similar study of these fungi. For this purpose the classification proposed by Castellani has been of value as a basis of comparison since the cultures here considered were of the genera Monilia, Endomyces and Cryptococcus.

In the white, sometimes chalk-like particles of the sputum of these patients, the spores were noted as fat-like globules varying in size from several to 10 or 15 microns in diameter. After washing in salt solution, cultures of these particles were made on Sabouraud's maltose agar or on lactose agar with a pH approximately 5.2. This reaction was conveniently obtained by adjusting the media with $\frac{N}{1}$ hydrochloric acid until it just turned brom-purple (dibromocresol-sulphonephthalein) to yellow. Growth took place at 30° C. in one or two days. The cultures exhibited a white creamy unwrinkled appearance and a yeasty odor was detected in the cultures from the patients, S. B., A. S. and S. M. In the cultures classified as Monilia, budding yeast bodies were present and broad mycelial septate segments were noted in the bottom of the slant; no asci were seen. In those designated as being of the genus Cryptococcus, the culture consisted almost entirely of budding yeast forms with little or no attempt at mycelial formation. In

*From the Clinical Laboratories of the Jewish Hospital, Philadelphia.

BIOCHEMICAL REACTIONS AFTER FIFTEEN DAYS' INCUBATION AT 35° C

CULTURE	GENUS	LITMUS MILK	GELATIN	ARAB-INOSE	DEX-TRIN	DUL-CITOL	GALAC-TOSE	GLU-COSE	INULIN	LAC-TOSE	LEVU-LOSE	MAL-TOSE	MANNI-TOL	RAFFI-NOSE	SACCHA-ROSE	SAL-ICIN
S. B.	Monilia	ACD		A	0	0	A	AGs	0	A	AGs	A	A	0	A	0
V. M.	"	ACD	0	0	A	0	0	AG	AG	AGs	AG	AG	AG	As	AGs	0
J. K.	"	AC	0	0	0	0	AGs	AGs	AGs	AGs	A	0	0	0	AG	0
S. M.	Cryptococcus	$\frac{AC}{alk.}$	+	AA	A	0	A	AG	A	A	A	0	0	A	AG	A
I. L.	Endomyces	AC	0	0	0	0	A	A	0	A	A	A	AGs	0	AGs	A
I. G.	Cryptococcus	AC	0	AG	0	0	AG	AG	0	A	AG	0	AG	AG	AG	AG
A. S.	"	AC	0	A	0	0	A	AG	0	A	A	A	A	0	A	A

Abbreviations in the table: A=acid; G=gas; s=slight; C=coagulation; D=decolorized; + (gelatin)=liquified; $\frac{AC}{alk}$ =acid, coagulated, later alkaline.

those grouped with the Endomyces, large numbers of spores were noted within mycelial elements and buds were given off at the sides; asci were present.

In the study of the fermentative reactions, the carbohydrates were made up in ten per cent solutions in distilled water and sterilized separately to avoid possible breaking up due to prolonged heating in the presence of bouillon. The ultimate concentration of the carbohydrates in the bouillon was one per cent. Brom-purple was used as an indicator. The results of these reactions are outlined in tabular form.

Intraperitoneal injections into guinea pigs of 2 c.c. of suspensions of these cultures in salt solution (approximately 50 million spores to 1 c.c.) after 24 and 48 hours, produced a slight exudate rich in eosinophilic cells and lymphocytes. Blood smears at the same time showed beginning eosinophilic granulation in many of the leucocytes, and also well developed eosinophiles. Eosinophiles were noted in great numbers in the sputum of the patients from which these cultures were derived and blood eosinophilia was a marked feature in these patients, ranging from 5 per cent to 30 per cent. It could not be said, however, that any constant proportion existed between the extent of the clinical and the experimental eosinophilia noted in the animals. This property of inducing eosinophilia is also noted in yeasts derived from other sources.

Demonstration of specific agglutinins by conducting macroscopic reactions with emulsions of spores and homologous sera did not yield conclusive results. However, in the case of S. M., the serum of the patient agglutinated the spores in dilution of 1 to 10, whereas control sera agglutinated only in dilution of 1 to 2. Complement-fixation reactions were inconclusive. The antigens were the water emulsions of cultures prepared after the methods outlined by Michel[2] in the study of Monilia psilosis. The antisheep hemolytic system was used in the test.

The patients from which these cultures were isolated did well when treated with iodides in the dosage of about 2 gms. a day or more. The injections of vaccines made from suspension of spores was given a tentative trial with fair results such as seen often with the use of bacterial vaccines. This procedure in a larger series would afford more definite information.

SUMMARY

1. Yeasts belonging to the genera Monilia, Endomyces and Cryptococcus were found in large numbers in the sputum of patients suffering from asthma and chronic bronchitis.

2. On the basis of biochemical reactions some of these are comparable to the yeasts, particularly Monilia, found in cases of bronchomycosis described by Castellani.

3. Production of eosinophilia in the peritoneal exudate and blood of guinea pigs after intraperitoneal injections was noted as a constant feature.

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LABORATORY METHODS

ADAPTATION OF SHAFFER'S TITRATION METHOD FOR BLOOD SUGAR TO CLINICAL USE*

By Howard D. Haskins, M.D., and William P. Holbrook, B.S.,
Portland, Ore.

SHAFFER and Hartmann have recently published a complete system of methods for estimation of sugar in urine, blood, milk, and other solutions.[1] In all of these methods a copper reagent is used which is similar to Benedict's. This is partly reduced by the sugar. The further treatment of the mixture causes iodine to be set free. The iodine is titrated with thiosulphate, and from the amount of iodine estimated the copper equivalent of the sugar can be calculated.

Our interest was attracted to the micro-blood-sugar method because it enables physicians to make the estimations without the expense of investing in a reliable colorimeter.

Folin's method is the only colorimetric method of estimating blood sugar that we are willing to recommend. We find that the results secured by Shaffer's method are just as accurate as those by Folin's method.

In addition to strongly recommending Shaffer's method, we wish to propose a modification that removes the one difficulty which those who are not expert chemists would encounter in using the method. The difficulty referred to is that of standardizing the thiosulphate. We have overcome this satisfactorily by standardizing the thiosulphate against the copper reagent, which is very easily done. We have proved that this modification does not cause inaccuracy in estimation. Our experimental results will be reported after the description of the method.

Preparing the Blood for Estimation.—Draw into the syringe a strong solution of neutral potassium oxalate (30 grams of oxalate dissolved in 100 c.c. water), empty the syringe and expel the excess so that only a film of solution is left. Draw at least 3 c.c. of blood from a vein and empty the syringe into a dry tube. With an accurate pipette measure 2 c.c. of the blood into a dry flask and add 14 c.c. of distilled water. When the blood is completely laked add 2 c.c. of 10 per cent sodium tungstate solution and finally 2 c.c. of 2/3 normal sulphuric acid solution. The volume of the liquid is now exactly 20 c.c. Cork the flask, shake well and let it stand 5 to 10 minutes.

If all the protein has been precipitated, the mixture will be brownish-red

*From the Department of Biochemistry, University of Oregon Medical School, Portland, Ore.
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in color instead of bright red, and only a trace of foam will appear on shaking. If the proper results have not been secured add 10 per cent sulphuric acid a drop at a time until satisfactory. (If more than 2 drops are required, the alkalinity of the stock solution of sodium tungstate must be reduced by treatment with a definite quantity of sulphuric acid.) Use small size filter paper for filtration. If the filtrate is not clear and colorless, return all the liquid (and precipitate) to the flask, treat it with more acid, shake well, and after a few minutes filter again. This method of freeing the blood of protein is exactly in accordance with Folin's[2] system of blood methods. What follows is Shaffer's special technic.

Technic of the Estimation.—Measure exactly 5 c.c. of filtrate into a large test tube and mix with it exactly 5 c.c. of the special micro-reagent. Plug the tube loosely with cotton and place it in a bath that is boiling actively. After exactly 15 minutes remove the tube and cool it at once under the tap. Then let it stand in a jar of cold water for 5 minutes. Add 5 c.c. of N/1 sulphuric acid, mix and let it stand 1 minute.

Titrate the liberated iodine by adding N/200 sodium thiosulphate as fast as drops can be counted (mixing well) until a distinct change is noticed, then add more slowly until the color becomes light yellow. Now add about 1 c.c. of 2 per cent starch solution and continue the titration cautiously but not too slowly until the characteristic starch-blue color is lost and only a pale copper-blue remains. When near the end-point determine the effect of each drop added.

Calculation.—In the special table (devised by us to correspond to our modification of Shaffer's method) the per cent of glucose in the original blood will be found opposite the c.c. of thiosulphate used for titration.

If the sugar content is greater than the highest in the table, dilute some of the blood filtrate with an equal volume of water and use 5 c.c. of this for the estimation, multiplying the results by 2.

REAGENTS*

(1) *Microcopper Reagent.*—Dissolve each constituent separately.

(a) Dissolve 40 gm. C.P. anhydrous sodium carbonate (or 47 gm. pure photographic monohydrated carbonate) in 400 c.c. warm distilled water.

(b) Dissolve 5 gm. C. P. copper sulphate (crystals that have not effloresced) in about 100 c.c. water.

(c) Dissolve 7.5 gm. pure tartaric acid in 100 c.c. of water.

(d) Dissolve exactly 0.7 gm. pure potassium iodate in 100 c.c. of water.

(e) Dissolve 10 gm. pure potassium iodide in 100 c.c. of water.

(f) Dissolve 18.4 gm. pure neutral potassium oxalate in 100 c.c. of water.

When each is dissolved, mix (c) with (b), and pour the mixture slowly (with stirring) into (a). Combine (d), (e) and (f) and pour this at once into the carbonate-copper mixture. Transfer to a measuring flask. Rinse all the dissolving beakers with small portions of water. When cooled to room temperature fill to the liter mark and mix thoroughly. Keep the reagent in a

*The Shaw Supply Co (of Portland, Seattle, and Tacoma) will supply any or all of the chemicals used for the method, including ready prepared solutions.

tightly corked bottle. A little sediment will be deposited, use the clear top liquid for estimations.

If the chemicals are pure, the microreagent run as a control will give the same titration whether heated in a bath 15 minutes or not heated. With each batch of reagent run one heated control and compare it with the unheated.

(2) *Standard Thiosulphate.*—Very dilute thiosulphate does not keep well. Prepare a stock solution of sodium thiosulphate a little stronger than decinormal (dissolve about 26 gm. in about 1 liter of distilled water) and let it stand 2 days.

Dilute exactly 5 c.c. to 100 c.c. in a measuring flask. Mix and use this to titrate 5 c.c. of the copper reagent (after adding 5 c.c. water and 5 c.c. of N/1 sulphuric acid) following the directions given above for the rate of titration. The titration will be less than 19.5 c.c. When duplicate titrations agree within 0.1 c.c. prepare a dilution of the thiosulphate such that exactly 19.5 c.c. will be required for the titration. For example, if the titration is 19 c.c. it will be necessary to dilute 5 c.c. of the stock solution to 102.6 c.c. (i.e., $\frac{19.5}{19.0} \times 100$). First dilute to 100 c.c. in a measuring flask then add the 2.6 c.c. with a reliable pipette, and mix well. This ratio of dilution should be determined by similar titration of a control once in two weeks. Prepare the dilute solution each day that estimations are made. This dilute solution is approximately N/200, and is the one referred to in the technic. Keep the stock solution in a brown bottle well corked, set away where it will not get warm, under these conditions it does not deteriorate.

TABLE I

PER CENT GLUCOSE IN BLOOD CORRESPONDING TO C.C. THIOSULPHATE USED FOR TITRATION

C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT
18.6	.036	16.3	.101	14.0	.162	11.4	.232
18.5	.039	16.2	.104	13.9	.165	11.2	.237
18.4	.042	16.1	.106	13.8	.167	11.0	.242
18.3	.045	16.0	.109	13.7	.169	10.8	.247
18.2	.048	15.9	.111	13.6	.172	10.6	.252
18.1	.052	15.8	.114	13.5	.175	10.4	.257
18.0	.055	15.7	.116	13.4	.177	10.2	.263
17.9	.058	15.6	.119	13.3	.180	10.0	.268
17.8	.061	15.5	.122	13.2	.183	9.8	.273
17.7	.064	15.4	.124	13.1	.185	9.6	.279
17.6	.066	15.3	.127	13.0	.188	9.4	.285
17.5	.069	15.2	.129	12.9	.191	9.2	.289
17.4	.071	15.1	.132	12.8	.194	9.0	.294
17.3	.074	15.0	.134	12.7	.197	8.8	.298
17.2	.076	14.9	.137	12.6	.200	8.6	.303
17.1	.079	14.8	.140	12.5	.202	8.4	.307
17.0	.082	14.7	.143	12.4	.205	8.2	.312
16.9	.084	14.6	.146	12.3	.207	8.0	.316
16.8	.087	14.5	.149	12.2	.210	7.6	.325
16.7	.089	14.4	.151	12.1	.212	7.2	.335
16.6	.092	14.3	.154	12.0	.215	6.8	.345
16.5	.095	14.2	.157	11.8	.220	6.4	.357
16.4	.098	14.1	.159	11.6	.226	6.0	.368

(3) *Starch Solution.*—This should be made once a week. We prefer

"soluble starch" but undoubtedly common corn starch could be used. Mix about 2 grams of starch with about 10 c.c. of water and pour it into about 90 c.c. of boiling water, mix and boil one minute. A few drops of toluol may be added as a preservative.

(4) *Standard Sulphuric Acid.*—The normal solution may be purchased if one is not used to checking standard solutions. For the ⅔ normal solution dilute 100 c.c. of the normal sulphuric acid with 50 c.c. of distilled water.

(5) *Sodium Tungstate Solution.*—C.P. tungstate should be used. The solution must be tested[3] for excess of carbonate as follows. Measure with a pipette 5 c.c. of the solution, add a drop of methyl orange solution and some distilled water, then titrate with the ⅔ normal acid until the yellow color changes to a slightly reddish yellow. Between 3 and 3.3 c.c. should be required. If the titration is greater add twice normal sulphuric acid (made by diluting about 11.5 c.c. C.P. acid to 200 c.c.) to the whole stock of 10 per cent sodium tungstate solution to bring its alkalinity down to the proper limits. Mix and titrate again.

TABLE II

COMPARISON OF ESTIMATIONS BY FOLIN'S AND SHAFFER'S METHODS

Blood	1	2	3	4	5	6	7
Folin	.070	.109	.117	.124	.130	.167	.222
Reagent I	.069	.106	.118	.124	.132	.165	.222
Reagent II	.072	.109	.123	.126	.134	.169	.220
Reagent III	.069	.108	.117	.122	.132	.167	.219

Note: Control titrations of Shaffer's reagents were as follows: I—19.55 c.c., II—19.3 c.c., III—19.7 c.c.

TABLE III

COMPARISON OF BLOOD SUGAR ESTIMATIONS BY SHAFFER'S ORIGINAL METHOD AND BY THE PROPOSED MODIFIED METHOD

Blood	1	2	3	4	5	6	7	8	9
Shaffer	.047	.072	.099	.102	.120	.129	.229	.338	.433
Reagent V	.048	.071	.100	.102	.122	.129	.230	.342	.436
Reagent VI	.045	.069	.101	.103	.123	.130	.231	.341	.431
Variation	.002	.003	.002	.001	.003	.001	.002	.004	.003

Note: Reagent IV used for the original method gave a titration of 19.6 c.c. The control titration of reagent V used for the modified method was 19.9 c.c. and of reagent VI—19.0 c.c.

DISCUSSION

Different samples of copper reagent do not always give exactly the same titration value when accurate N/200 thiosulphate is used. We find that this value is generally close to 19.5 c.c., therefore, we have taken this figure as the average titration value. Varying the amount of iodate in the reagent changes the titration value.

Estimations by Shaffer's method using accurate reagents are practically identical with those by Folin's method (see Table II). Also when we used copper reagents that had a slightly different titration value (using exact N/200 thiosulphate the control estimation on reagent 2 is 19.3 c.c., and on

reagent 3 it is 19.7 c.c.) and made the thiosulphate correspond to the reagent in each case in accordance with our modification of the method. The results agreed very closely with the estimations both by Folin's method and by Shaffer's (with accurate reagents).

By changing the iodate content we secured copper reagents having a titration value differing more widely (19.0 c.c. and 19.9 c.c.). With these less accurate reagents we obtained very satisfactory results by using our modification of the method (see Table III). In most cases the variations from the accurate estimations by the original method were only 1 to 3 mg., and the greatest variation was 6 mg. Most of the variations were of the same order as were secured by running duplicate or triplicate estimations by any one of the methods. The Folin duplicates which we ran, differed by 1. to 2.3 mg. Shaffer triplicates varied by 0.5 to 4.5 mg. These results convince us that our modification does not perceptibly affect the accuracy of the Shaffer method.

The explanation for such good results with slightly inaccurate reagents is, undoubtedly, that the variation of the thiosulphate from a true N/200 solution compensates for the deviation of titration value of the coper reagent from the average value. For example, if the reagent is a little weak, then the thiosulphate solution prepared for use with it will be more dilute than N/200, thus avoiding the undertitration that would occur in a sugar estimation if an exact N/200 solution were used.

SUMMARY

An easy method of standardizing the thiosulphate is proposed as a modification of the Shaffer method, the thiosulphate being checked against the copper reagent.

Because of this adjustment of the solution the control estimation of the reagent is always 19.5 c.c. of dilute thiosulphate.

This constant titration value has enabled the authors to construct a table from which the per cent of glucose in the blood can be read without calculation.

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AN AERATION APPARATUS FOR THE DETERMINATION OF UREA IN BLOOD*

By Armada T. Weathers, B.Sc., and H. C. Sweany, M.D., Chicago, Ill.

THE same difficulty mentioned by Boggs and McEllroy[1] in the distillation of ammonia formed by urease in the determination of urea in the blood by Folin and Wu[2] technic was also encountered in our work. A method similar to that described by Rakestraw[3] was then used with excellent results. As we perform a great many urea and nonprotein nitrogen determinations in this laboratory, we were compelled to devise a different means of separating the ammonia from the original digest than by the ordinary distillation method, where only one sample can be distilled at a time requiring the full time and

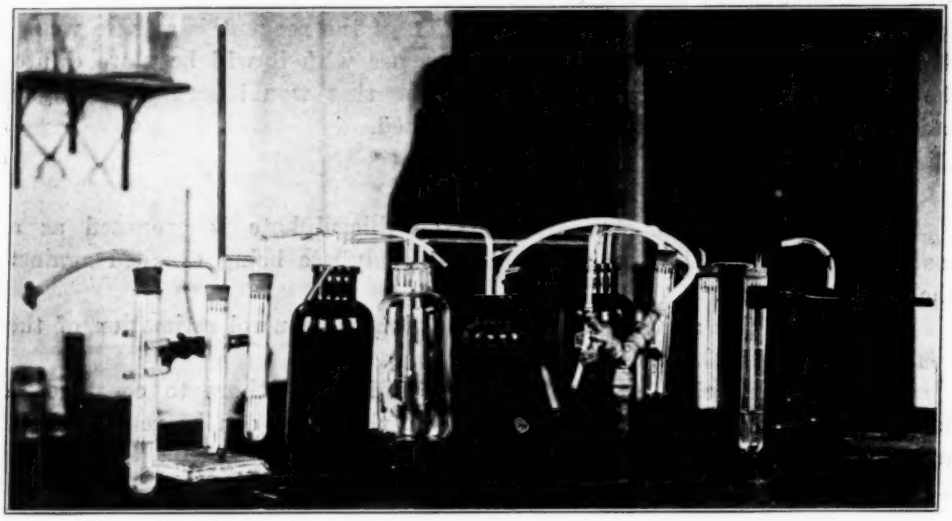

Fig. 1.

careful attention of one technician. The idea of designing an apparatus to run eight or more determinations simultaneously by aeration was decided upon in order to save this extra time and labor.

A very simple aeration apparatus was then devised. (Figs. 1 and 2.) It is operated by positive air pressure and requires practically no attention. This apparatus may be made from materials available in any hospital or student laboratory, viz.: test tubes, rubber stoppers, large, wide-mouth reagent bottles, glass, and rubber tubing and Hofmann screw clamps. The apparatus may be dismantled when not in use, does not require the cumber-

*A Simple and Inexpensive Method whereby eight samples may be run at one time. From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois.

Received for publication, March 20, 1923.

some pipes that are frequently used, and insures a dry ammonia-free air current for aeration. Fig. 3 shows the aeration unit. Four of these may be attached to bottle 3 and four to bottle 3′ so that eight samples may be aspirated simultaneously. In Fig. 2, bottle 1 contains sulphuric acid, bottle 2 is full of glass beads and is used to catch any sulphuric acid that may come over. Bottles 3 and 3′ contain cotton to break the air currents and to take out the last traces of the acid.

After the digestion of the blood filtrates with urease in (25 × 150) test tubes instead of (25 × 200) as described by Folin and Wu,[2] 2 drops of paraffine oil are added and the test tubes attached to the aeration units as in

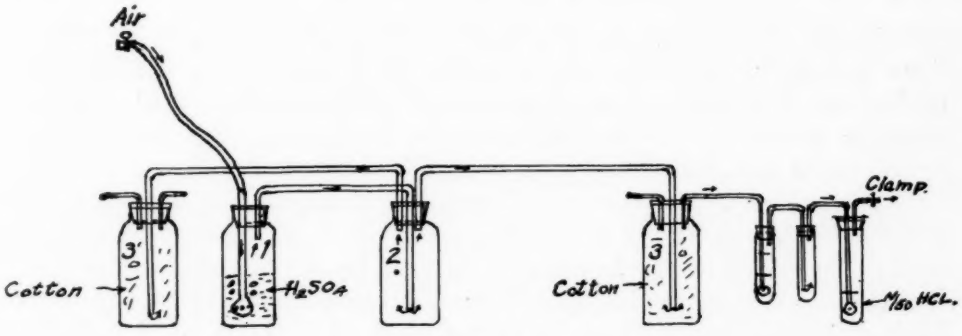

Fig. 2.

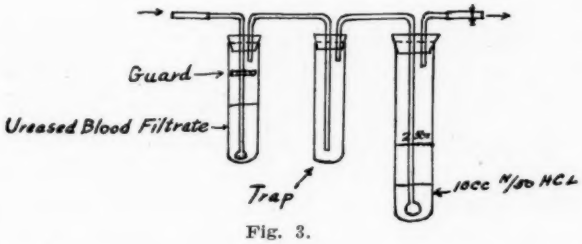

Fig. 3.

Fig. 3. Then 2 c.c. of 10 per cent NaOH are run through the tube, washed down with ammonia-free water and the unit connected to the apparatus. Thirty minutes is sufficient to drive over all the ammonia. During the first 15 minutes the air is passed slowly enough to give 2 bubbles per second. In the next 10 minutes the rate is increased so that 4 bubbles pass in the same time, and in the last 5 minutes the air is adjusted to give about 10 bubbles per second so that the last traces of ammonia may be swept out. Thus eight blood filtrates may be digested, aspirated, Nesslerized, and read in the colorimeter in one hour. Urea determinations on the same blood filtrates have been made by both the distillation and the aeration methods. The results of Table I indicate that a fair agreement between the two methods can be obtained.

This apparatus may also be used for nonprotein nitrogen determinations. It was found that the direct Nesslerization method of Folin and Wu[2] for nonprotein nitrogen gave cloudy solutions at times that could not be

TABLE I

CASE NO.	MG. PER 100 C.C. OF BLOOD				REMARKS
	UREA N BY DISTILLATION	UREA N BY AERATION	NONPROTEIN N.	AMINO-ACID N.	
15,817	9.95	10.00	26.2	7.0	Incipient T. B.
11,077	10.25	11.10	29.5	8.0	Far Advanced "
10,634	13.96	13.98	31.8	7.1	Incipient "
15,137	12.20	11.84	29.2	7.8	Far Advanced "
16,350	13.90	14.20	35.5	7.4	Incipient "
15,991	12.00	10.80	29.4	7.8	Far Advanced "
16,293	16.30	15.80	39.2	7.5	Far Advanced "
16,355	9.10	9.00	25.8	6.9	Incipient "
Sedg. 18123	11.14	10.20	26.9	8.0	

read in the colorimeter. As we run 15 to 40 determinations on each blood, there is usually no filtrate left to repeat the determination. So the Folin method was discarded and a microchemical Kjeldahl method used to decompose the organic matter in the Folin and Wu filtrate. The ammonia was set free with NaOH and aspirated as described above for urea.

This technic was then extended to the determination of ammonia, urea and total nitrogen in the urine. The ammonia is determined by Steele's[4] method up to the point of aeration, the urea by the method of Van Slyke and Cullen,[5] also up to the point of aeration, and the total nitrogen by the Bock and Benedict's method[6] up to the point of distillation. All three of these determinations may then be completed according to the aeration method described above.

The general use of this technic in routine work for microchemical nitrogen determinations, as a time- and labor-saving device, is due to the fact that many determinations can be made at the same time with practically no attention required during the process of aeration.

If the laboratory has no pressure line, a vacuum line or suction filter pump may be used. In this case, the aeration units must be reversed and the laboratory freed from all ammonia fumes. However, it is best to connect small wide mouth absorption bottles, containing sulphuric acid to each aeration unit. If it is desired to make more than eight determinations simultaneously, two more bottles containing cotton and designed as 3 and 3′ may be connected to bottle 2. By this addition 16 determinations may be run at one time. In this case it is better to make at least 3 standards as the blood urea nitrogen should be Nesslerized as near the same time as the standard.

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A STUDY OF THE AMMONIA PRESENT IN GASTRIC CONTENTS FOLLOWING TEST MEALS*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

INTRODUCTION

CONSIDERABLE work has been published concerning the ammonia content of the gastric juice in the fasting state. On the other hand more data bearing on the ammonia content of the gastric juice in normal and diseased states is needed.

Bidder and Schmitt[1] in 1852 reported on the absence of ammonia from the gastric juice of man. Leo[2] noted a trace present in health and increased amounts in uremic cases. Rosenheim[3] discovered a trace of ammonia and thought it came from the gastric secretion, or from the decomposition of protein. Martius and Lütteke[4] found ammonia present only in cases of uremia. Ewald[5] found ammonia in 50 per cent of the cases examined. Strauss[6] found ammonia in eighty per cent of the cases he examined. Sticker[7] following test meals found ammonia in gastric contents, and thought it came from the saliva or from the decomposition of protein. Sommerfeld[8] could find no trace of ammonia in stomach contents of a man with a gastric fistula. Zunz[9] found small amounts of ammonia present and found it increased in cases of malignant disease of the stomach. Carlson,[10] in a man with a gastric fistula and in the contents removed by Rehfuss tube, found ammonia in all cases. The amount was from 2 to 3 mg. to each 100 c.c. of gastric juice of a man. Huber[11] found ammonia present in the gastric juice of a man in small amounts. It varied markedly in different individuals, and was increased by a high protein diet and by the addition of ammonium salts to the food. He also noted that the fundic mucosa contained the most ammonia, the pyloric next, and the cardiac end of the stomach contained the least and that the duodenal mucosa contained less ammonia than the fundic mucosa. He noted that in certain pathologic conditions especially gastric ulcer and cancer that the amount of ammonia in the gastric juice is definitely increased, but this increase may not be greater than that found continually in normal persons.

This paper contains a study of the ammonia content of the gastric contents removed one hour after an Ewald test meal, which consisted of two ounces of bread, and 200 c.c. of water. The ammonia was estimated by Folins[12] aeration method using n/50 acid. The total acidity, free acidity, loosely combined acid, and the organic acid and acid salts were estimated by Töpfers method.

Table I contains the results obtained in the study.

*Received for publication, February 26, 1923.

TABLE I

SHOWING AMMONIA AND ACID CONTENT OF GASTRIC CONTENTS

CASE NO.	TOTAL ACIDITY	FREE ACID	LOOSELY COMBINED ACID	ORGANIC ACID AND ACID SALTS	AMMONIA MILLIGRAMS PER 100 C.C. CONTENTS	DIAGNOSIS
1	40	20	10	10	0.6	Normal
2	50	18	20	12	2.1	"
3	60	20	—	—	4.6	"
4	46	18	—	—	0.9	"
5	50	20	—	—	2.0	"
6	48	18	—	—	3.5	"
7	45	16	—	—	3.0	"
8	55	22	—	—	2.5	"
9	50	18	—	—	3.6	"
10	46	24	—	—	5.6	Gastric ulcer
11	75	18	24	28	8.4	" "
12	38	0	14	24	1.4	Chronic appendicitis
13	70	0	50	20	1.9	" "
14	20	0	—	—	1.6	" "
15	54	—	—	—	1.5	" "
16	50	0	32	18	2.1	Gall stones
17	40	0	26	14	0.9	" "
18	24	0	—	—	4.2	Diabetes
19	46	24	18	4	1.9	"
20	130	50	55	25	1.8	Duodenal ulcer
21	72	28	26	18	1.4	" "
22	20	0	10	10	1.2	Cirrhosis of liver
23	65	30	25	10	1.1	Pyloric adhesions
24	68	50	13	5	1.4	Cholecystitis
25	55	25	25	5	4.8	"
26	52	28	10	14	3.5	"
27	30	15	10	5	7.0	Gastric cancer
28	66	15	30	20	0.8	" ulcer
29	15	0	10	5	1.5	Pernicious anemia
30	65	35	10	10	1.7	Secondary "
31	84	70	12	2	0.5	Duodenal ulcer
32	66	20	—	—	0.6	" "
33	84	32	—	—	1.2	Chronic appendicitis
34	75	20	—	—	1.5	" "
35	60	18	—	—	2.7	" "
36	80	22	—	—	3.0	Gastric ulcer
37	95	30	—	—	2.5	" "

CONCLUSIONS

1. The ammonia content of the stomach contents following Ewald test meal varies from 0.6 mg. per 100 c.c. of contents to 4.6 mg.

2. There does not seem to be any diagnostic significance to the ammonia content of the gastric contents following a test meal.

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AN UNSUSPECTED SOURCE OF ERROR IN THE GRAM STAIN*

By Wm. M. Sheppe, M.D., and Mary G. Constable

INTRODUCTION

DURING and immediately following the period of the World War, laboratory workers experienced great difficulties in obtaining reliable biological stains. This condition was especially manifested in the difficulty encountered in making satisfactory Gram stains.

Many private investigators, supplemented by committees of the American Society of Bacteriologists, studied numerous samples of American stains, particularly gentian violet, with the idea of determining their relative dependability.

Modifications of technic involving changes in staining times and types of decolorizers were advocated by Burke[1] and others. The former advised the addition of a pinch of sodium bicarbonate to the gentian violet on the slide but was unable to explain the increased reliability of the stain brought about by this simple procedure. This fact is of interest in view of our subsequent findings.

This laboratory was not exempt from the difficulties encountered elsewhere in making satisfactory Gram stains. Using the same stains and the same organisms, results varied with different students and with the same student from day to day. The chief difficulty lay in the too easy decolorization of Gram-positive organisms. Following the recommendations of the committee on the Descriptive Chart, several different samples of gentian and methyl violet were employed and the technic recommended by the committee rigidly adhered to. Some improvement was noted but annoying failures of Gram-positive organisms to retain the violet stain continued to occur and further search for the source of error was manifestly in order.

In considering the problem the authors were guided by the following facts:

1. Acids are very active in the removal of stains from bacteria.

2. The good results obtained by Burke after the addition of an alkali to the gentian violet may have been brought about by the neutralization of some unsuspected acid.

3. So far as we could determine, no investigation has been carried out on the properties or possible variations in chemical composition of the iodine solution.

Working from the above postulates a series of experiments was carried out along the following lines:

*From the Laboratory of Bacteriology and Pathology University of Virginia.
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PROCEDURE

I. Freshly made Lugol's solution, prepared according to the standard formula, was found to be neutral to litmus. This solution was used in the experiments listed under II and III.

II. Fifty c.c. of Lugol's solution were placed in each of four flasks and subjected to the following conditions:

Heat (Incubator 37° C.).
Heat and Moisture (Moist air at 37° C.).
Sunlight.
Cold (18° C.).

At the end of five days no change had occurred in any of the flasks.

At the end of ten days the following results were noted:

Flask No. 4 (exposed to cold) showed no change. The solutions in flasks 1, 2 and 3 were slightly acid to litmus. On the addition of a little sodium bicarbonate a definite evolution of gas took place. The color of the solutions was much lighter than when first prepared.

Smears were made from 24-hour agar cultures of Staphylococcus aureus. Gram stains were made on these smears using the standard technic but substituting the solutions from the flasks for the fresh Lugol's. Stains made using solutions from flasks 1, 2 and 3 were uniformly Gram negative. Flask 4 yielded a positive.

The above procedure was repeated three times, the results being the same each time.

III. To each of the four flasks of Lugol's solution, concentrated Hydriodic acid was added in the strengths of .25, .5, .75 and 1 per cent respectively. Smears were made and stained as above, except that the acidified Lugol's solution was added.

Results:	No. 1 (.25 per cent)	Organisms	Gram positive.
	No. 2 (.5 per cent)	"	" amphophile.
	No. 3 (.75 per cent)	"	" negative.
	No. 4 (1 per cent)	"	" negative.

IV. The Lugol's solution in use by a student who was obtaining false Gram negatives was obtained. Stains made with this solution were all Gram negative. The reaction of the solution was acid and the addition of a pinch of sodium bicarbonate resulted in a strong evolution of gas. Smears made with this neutralized solution were uniformly positive. This procedure was followed in the case of other students who were unable to make satisfactory stains and the difficulty in each instance was immediately overcome.

DISCUSSION

The results of the investigation recorded in II show that the originally neutral Lugol's solution when exposed to the proper conditions is capable of undergoing a chemical change resulting in acid formation and that the amount of acid formed is quite capable of decolorizing Gram-positive organisms.

Bordier[2] has shown that this reaction takes place very readily when dilute solutions of iodine in water are exposed to light. The reaction is as follows:

$$I_2 + H_2O \leftrightarrows HI \text{ (Hydriodic acid)} + HIO \text{ (Hypoiodus acid)}.$$
$$HIO + O_2 \rightarrow HIO_3 \text{ (iodic acid)}.$$

Bordier believes that iodine does not form a true suspension in water but is in a colloidal suspension. The particles of iodine in suspension being in the colloidal state bear electrical charges and tend to act like large ions. Therefore the chemical properties of the electrically charged particles are not exactly identical with those of free iodine and hence will not combine with water to form the acid. However under the action of the violet and ultra-violet rays the electrical charges are removed from the ions. These particles thus discharged acquire their original chemical properties. They are then able to combine with the hydrogen of the water to form hydriodic acid.

The reaction, it will be noted, is a reversible one and this agrees with our experience as the solutions on further standing lost a part or all of their acidity. We think that this perhaps explains the variability of results obtained by a given student at different times.

The experiments recorded in III show that hydriodic acid is a powerful decolorizer of Gram-positive organisms acting in strengths as low as .5 per cent (pH 3). In IV the facts demonstrated are put to the practical test of experience and are found to satisfactorily correct the preexisting error.

It seems to us entirely possible that the good results obtained by Burke by adding an alkali to the gentian violet resulted from the neutralization of an acid Lugol's solution.

The light from individual desk lights, augmented by heat from steam sterilizers and bunsen burners is quite sufficient to inaugurate the reaction. The question arises at once, why is this discrepancy resulting from an acidification of Lugol's solution of a comparatively recent date? This we cannot answer conclusively but offer the following as possible explanations.

1. The old Grubler stains were possibly alkaline and so neutralized any acid formed in the iodine solutions.

2. Until about three years ago no artificial light was employed in the laboratory for microscopic work. The iodide solutions on the desks were therefore subjected to a very mild diffused daylight. With the introduction of the individual desk lights, the solutions were subjected to the action of the intense rays from the Mazda bulbs with which these lights are equipped. This light is not only of great intensity (which is probably of prime importance) but is rich in the light waves occurring toward the violet end of the spectrum. Bordier believes that these actinic rays are probably responsible for the inauguration of the reaction.

The second hypothesis is regarded as the most tenable explanation of the fact that the Lugol's solution in the laboratory was stable until the introduction of artificial light.

SUMMARY

1. An error in the Gram-staining technic, resulting in the decolorization of Gram-positive organisms developed. This error was not corrected by changes in procedure or the use of other samples of gentian violet.

2. The Lugol's solution in use was found to have become acid. This acidity could be produced at will in test samples of the iodine solution by heat or light.

3. Stains made with these test solutions were regularly Gram negative as were those in which small amounts of hydriodic acid were added (.75 per cent).

4. Neutralization of the acidity by sodium bicarbonate resulted in the disappearance of false Gram-negative stains.

5. It is recommended that Lugol's solution be kept in dark bottles in a fairly cool place.

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EDITORIALS

Insulin Therapy

TOWARD the end of June, insulin was released for use by those physicians properly equipped with adequate hospital and laboratory facilities who are willing to assume the responsibility for its use. Prior to this a report was made in the *Journal of the American Medical Association* by the Insulin Committee, outlining the general procedure of treatment. Several independent articles have already appeared and an avalanche thereof may be anticipated. In the meantime a comprehensive symposium of articles has been published in the latest number of the *Journal of Metabolic Research*. It covers 438 pages and is an unusually valuable group of communications. Since the Journal is not available to the majority of physicians, it may be worth while to recapitulate the more important conclusions of the various authors.

Route of Administration.—The extract is as a rule given subcutaneously. An exception is found in severe acidosis and in coma, where the intravenous method is preferred. Joslin, Gray and Root gave insulin by mouth on two occasions when the patients had previously been rendered sugar free

by subcutaneous inoculation. Sugar rapidly reappeared in the urine and acidosis returned. Both disappeared upon return to subcutaneous administration. Woodyatt has given the drug through the mouth, rectum and vagina, intranasally and by inunctions. Positive effects were obtained only with subcutaneous and intravenous injections, while the results with other methods were either very weak, doubtful or frankly negative.

Local Reaction.—Early in the isolation of insulin the high salt content of the pancreatic extracts caused a stinging or burning at the site of injection, which persisted for an hour or more. This local reaction was rapidly eliminated but even now certain batches of extract have a similar effect lasting upwards of ten minutes. Some local tenderness follows treatment with occasional slight induration, but not as great as that following the hypodermic administration of many other drugs. Joslin reports that in over 5,100 injections no abscesses have developed. Induration has been fairly frequent and in one patient there developed a small crusted ulcer without evidence of infection which may have resulted from irritation of the tricresol. Wilder reports severe local injury with necrosis and sloughing of the skin in a few instances. One child developed a necrotic area ten by four centimeters on the thigh, which may be an unsuitable site for injection because of the tightness of the tissues. This also was attributed by dermatologic consultants to tricresol action.

Williams reports only one serious local reaction, which occurred with early extract before its purification had been perfected. He noted sterile abscesses in two others among several thousand injections. Fitz and the workers at the Brigham Hospital observed a local urticarial reaction in one instance, the extract used being one of the early isolations. Cutaneous tests with the protein of beef, pork and horse serum as well as with insulin failed to show any evidence of sensitization. Woodyatt avoids unnecessary pain, soreness, scarring and necrosis by using very small gauge needles (27-29) with a three-fourth to one inch length, inserted full length under the skin into the loose areolar tissue. He warms the solution before injection and takes particular care not to produce a lump during administration. Injections are usually given into the thigh. Allen and Sherrill have never had an infection or even a marked local reaction with the later extracts and remark on the fact that not a single infection has occurred among the patients giving their own injections at home.

General Reaction.—The most important general reaction is the development of hypoglycemia. This will be discussed under a separate heading. Joslin and his coworkers observed protein reactions in four patients; Williams has seen two anaphylactic reactions, and Wilder three. Geyelin observed serum sickness in three of nine cases and found it necessary to desensitize two with small doses of insulin. These reactions were all observed before the extract was more successfully purified and none have been reported from the later products.

Frequency of Administration.—Banting, Campbell and Fletcher have found that in the average case the injections need not be given three times

a day. When the insulin requirement is small it may be taken in one dose before a meal in which the greater part of the total carbohydrate has been concentrated. This does not maintain a low blood sugar level. As a rule, two doses daily, one before breakfast and a second before the evening meal, are preferable. The dividing of doses in this way allows a greater utilization of food stuffs per unit of insulin.

Joslin, Gray and Root feel that it is as yet too early to state definitely whether two injections daily would be as efficacious as one given before each meal. They recognize the advantages from the patient's viewpoint of administering the drug but twice daily. Williams remarks "the time and frequency of dosage are important. Apparently the action of extracts lasts only a few hours. In severe cases it should be administered twice or better three times a day in properly spaced doses." Wilder and his associates believe that maximal results were obtained with single injections of the entire amount thirty minutes before breakfast, and with food distributed equally between the three meals. This was particularly so when the dosage did not exceed thirty insulin units. As a rule, the second meal comes early enough to neutralize any severe late effect on the glycemic level. The danger of hypoglycemic reaction may be minimized by careful balancing of the diet, and should symptoms arise, by prompt administration of small amounts of sugar. They favor a single daily injection not only because of the greater ease in treatment, but also because individual cases who were treated by both methods have appeared to do better with single injections.

Woodyatt has in his work practically eliminated multiple daily doses and in ninety per cent of all cases, administers a single morning injection. Rarely he gives two doses a day. This of course does not apply to complications such as acidosis or coma.

Allen and Sherrill prefer divided doses with two or three per day, even when the dosage has been as low as one and two units per day. In the severe types they prefer three injections. Occasionally with three daily administrations, the blood sugar level remains satisfactorily low during the day but rises in the night so that by morning glycosuria exists. Here they prefer to give the extract four times daily at six hour intervals with the feedings appropriately arranged.

Dosage.—The average unit is considered as metabolizing 1-1.5 grams of glucose. Woodyatt has seen cases in which one unit reduced glycosuria by as much as three grams. In mild arteriosclerotics and renal diabetics on the contrary, insulin exerted little effect. The ratio varies in practical work with the individual patient and particularly with the extent of glucose retention. Thus the value of each unit per gram of exogenous glucose is higher after the patient has been thoroughly treated than at the beginning when endogenous stores were present in excess. However, for the starting of treatment, the above ratio may be safely accepted. If after one or two days of observation a patient has been found to excrete forty grams of glucose in twenty-four hours, twenty-five units of insulin may be prescribed for the first day's dose.

All writers stress the importance of individualized dosage and the avoidance of hypoglycemia. We shall see that there is some divergence of opinion regarding the necessity for maintaining complete aglycosuria. All have found that in acidosis or coma, or with superimposed infections, the dose required to render a patient sugar free and to remove the hyperglycemia is greater than without such complications. When at the beginning of treatment the blood sugar is high and there is a consequent larger concentration in the tissues, larger quantities of insulin are required to render the patient sugar free than is later necessary to keep him so. Thus in one case after four days, insulin was reduced from sixty to forty units, the total glucose intake remaining the same. Joslin, Gray and Root give the average daily number of units in fifty-three patients as eleven. They find no evidence at present that the dosage must be increased provided we disregard an increase early in treatment, to care for the constantly increasing diet, and allow for increased metabolic needs after the undernutrition has been relieved. Wilder and his coworkers vary the dose according to the patient's basal tolerance and with the diet. In general they provide a food allowance of 2000 to 2600 calories. As a rule between ten and thirty insulin units will be needed to raise the tolerance sufficiently to permit this desired higher caloric value. They give as high as thirty units in one dose.

Those working at the Presbyterian Hospital in juvenile diabetes have given somewhat larger doses with higher total caloric intakes, so that the children may gain weight more rapidly. Woodyatt has developed a relatively simple system of dosage determination. On a basal diet he establishes a daily excretion of sugar. Thus if the individual excretes steadily twenty-five grams of glucose, twenty units will about render him sugar free. He then receives one-half of this calculated dose. This dose is increased by five units daily, and later the caloric intake is gradually increased to the desired amount. Allen and Sherrill state that the dosage for regular use ranges between four and seventy units per day. Under any plan compatible with fair strength and comfort, it is seldom possible to treat a really severe case with less than twenty units per day. Several of Allen's cases received between thirty and fifty units while a maximum of seventy units was reached in only one case.

Hypoglycemia.—The reaction of greatest importance following insulin results from too rapid or too great fall in blood sugar level with resulting hypoglycemia. Fletcher and Campbell found that a low level was reached anywhere from two to twelve hours after a single administration. The glycosuria threshold is usually around 170 mg. per 100 c.c., but has been observed as high as 300 and as low as 100. The higher the initial blood sugar readings the more rapidly does the percentage tend to fall. There is no definite correlation in all cases between the amount of insulin administered and the degree of fall even though the initial blood sugar be the same. In diabetes the blood sugar is easily lowered to the normal range but is somewhat less easily reduced below this.

The symptoms of hypoglycemia consist of excessive hunger, nervousness,

tremulousness with sometimes actual tremor, and at times, a feeling of weakness or a sense of goneness. In children the pulse may increase while in adults sweating becomes an outstanding feature in the more profound hypoglycemias. As the condition progresses the nervousness may become definite anxiety, excitement or even emotional upset. Syncope or collapse may occur but convulsions have not been observed.

Some patients become aware of hypoglycemia when the blood sugar is between .08 and .09 per cent. Others experience no symptoms with levels as low as .054. A severe reaction has been observed with a blood sugar percentage of .060 per cent and again a mild one in an individual with a concentration of .040 per cent. A blood sugar percentage of .035 is usually accompanied by unconsciousness. Reactions are generally more severe and more prolonged in the extremely undernourished. Even when there has been an accurate balancing of insulin and carbohydrate intake, reactions occasionally occur, due presumably to some unusual delay in the absorption of food. Reaction usually occurs two hours after administration but may be delayed, and should be particularly avoided at night when the patient may be asleep. Joslin observed thirty reactions in the course of 5,153 injections. These occurred in eleven individuals. The smallest dose causing hypoglycemic symptoms was one insulin unit. This occurred in a man with tolerance for 114 grams of carbohydrate, but who was weakened by diarrhea. Allen has observed a reaction after one-half unit in an individual extremely weakened and emaciated, on a low diet. He observes that heavy exercise increases the tendency to hypoglycemia.

The Toronto observers particularly found that reactions were prone to occur when changing to a new batch of insulin, the unit value of which might be slightly greater than that which had been previously used by the patient. This variation in the strength of batches has been practically overcome by Eli Lilly and Company. Woodyatt and Wilder standardize their new batches of insulin by clinical tests on appropriate patients.

Treatment consists in the administration of glucose usually by mouth. Orange juice is very successful. Other sugars than glucose are not as highly recommended. If necessary, glucose may be administered intravenously. Other substances such as hot tea or coffee and beef extracts produce temporary relief from symptoms but no increase in blood sugar concentration and are invariably followed by relapse. As a rule there is no recurrence after the administration of glucose. Often five grams of glucose will tide a patient along till the next meal. Twenty grams are frequently administered.

The administration of epinephrin is recommended in unconsciousness. This apparently causes a mobilization of tissue sugars into the blood. Such treatment must be followed by the giving of orange juice or some preparation containing a moderate quantity of dextrose. Allen reports one unusually severe case who did not respond to epinephrin but who revived immediately following an intravenous injection of glucose. The possible interpretation was that the carbohydrate stores in the tissues had already been too thoroughly exhausted. Allen has found that in emergency, sterilization

for intravenous glucose administration is not indispensable, for a clean filtered solution injected intravenously does not cause infection.

The Toronto workers advise that each patient experience a mild hypoglycemic reaction while still under hospital observation so that he may more readily recognize the symptoms should they occur under home treatment.

Acidosis and Coma.—In impending coma and actual coma the amount of insulin administered must be decidedly higher than in the less severe cases. The drug may be given intravenously and if large doses are administered, glucose should be given at the same time. Ketone bodies appear to be actually burned or synthesized into some nontoxic body, possibly carbohydrate in nature. At all events they are disposed of in the presence of large amounts of insulin without corresponding changes in the urine or expired air. Not all coma cases recover with insulin, probably because of permanent toxic damage to the nervous system or other tissues. If this be true early administration is essential and the intravenous method is preferable. In some of the earlier cases such as two reported by Williams, death was due to insufficient insulin. The majority of those who have progressed to a fatal outcome were suffering from complicating conditions such as influenzal pneumonia, moist gangrene, etc. Carbohydrate administration prevents hypoglycemia, furnishes energy, reduces the incomplete combustion of fat and protein, thus limiting ketone production, and finally, aids in the combustion of those ketones already present in the blood and tissues.

Allen and Sherrill outline a plan of treatment to be followed in precoma. In actual or threatened coma a blood sample is taken and 25 units of insulin are injected into the vein through the same needle. An additional dose of from 25 to 50 units is immediately given subcutaneously. Further subcutaneous doses follow at intervals of one or more hours, depending upon the laboratory findings. Glucose is given simultaneously so that the blood sugar concentration may be maintained above 300 mg. per 100 c.c., while the acid bodies are being cleared up as rapidly as possible. At least 100 units are usually required in a dangerous coma case. Other authorities repeat the intravenous dosages.

Death in coma under insulin has been variously ascribed to hypoglycemia, infection, and unknown toxic properties of the drug. There is some evidence that extremely high dosages of the latter do possess some toxic action. More probably death in unsuccessful cases is due to irreversible protoplasmic injuries which result from acidosis, but are not removed by removal of the latter.

Administration of Alkali.—Alkali therapy when used in conjunction with insulin appears to have considerable value in coma. To be sure, excess of sodium bicarbonate does harm and this accounts for the firm stand of a number of clinicians against its use. The Toronto observers found that while insulin diminished the extent of acidosis to a remarkable degree, the alkali reserve remained at a very low level in spite of reduction in blood acetone bodies. If acidosis has been overcome, alkali may therefore be administered to cause a return to normal of the alkali concentration in the blood, with

definite clinical improvement. Alkali should be given as early as possible. Since the absorption of substances introduced into the stomach during coma is most erratic, intravenous administration is preferable. Campbell suggests from the work of Palmer and Van Slyke that one might safely introduce 20 grams of sodium bicarbonate per 84 pounds body weight.

Woodyatt in treating precoma institutes a diet containing 0.5 to 0.75 grams protein and 2.0 to 2.5 grams fat per kilogram with a low carbohydrate ratio and gives the patient at once 20 grams of sodium bicarbonate by mouth. The base is repeated at the rate of 15 to 20 grams per hour until the blood plasma shows a reading of 40 to 45 volumes per cent CO_2. When insulin is used effectively he usually discontinues the soda after giving from 20 to 60 grams. Allen and Sherrill remark that any signs of acidosis should call for complete exclusion of fat and practically a diet of carbohydrate alone. They recommend sodium bicarbonate in five gram doses by mouth up to 20 to 50 grams in 24 hours (rarely in small doses intravenously).

Complicating Infections.—Opinion is unanimous that the high nutritive level made possible by insulin and the decrease in hyperglycemia are of decided benefit in complicating infections. Diabetes with tuberculosis may now be treated with more hope of success. The results with other medical and surgical infections, particularly gangrene, have been fairly satisfactory. The general resistance and the healing of wounds appear to be no better under insulin than they have been heretofore under efficient dietary control. However the results are decidedly better than when dietary control was not complete and benefit is particularly seen in the complicating acidosis. The lowering of tolerance which customarily occurs in infection may be combated by increase in the insulin dosage. Joslin remarks that general infections were uninfluenced by insulin save that complicating acidosis was lowered. Local infections appeared to heal more rapidly.

Optimal Diet.—All observers are agreed that the diet even under insulin should, particularly in adults, be no more than sufficient for the patient's metabolic needs. No attempt should be made to fatten patients needlessly. The diet theoretically may be raised to any desired amount, provided sufficient insulin be administered and provided the proper balance of protein, fat and carbohydrate be maintained. The diet should not be increased beyond such levels as will satisfy reasonable normal demands for energy. There are several reasons for this. Increased caloric intake causes increase of metabolic rate. Increased intake necessitates more insulin and more frequent injections with consequent greater liability towards hyperglycemia and also towards hypoglycemic reactions. Furthermore, if the patient who has attained a certain degree of obesity suddenly finds himself unable to procure his insulin, there is great danger of resultant acidosis. Extreme undernutrition is no longer necessary but moderate undernutrition still appears preferable to hypernutrition. The observers at the Mayo clinic aim to provide in general a food allowance of about 2,000 to 2,600 calories.

Williams records that the feeding of high diets and attempts to make their utilization possible by large amounts of insulin has not been satisfactory.

The best treatment apparently is a diet which will enable the patient to meet the energy requirements of light work with sufficient insulin to insure the metabolism of the food. Geyelin, working with children has attempted to bring the weight up to the normal for their age and height. The gain in weight ranged from two pounds in three months to 22 pounds in seven months. There was a corresponding increase in muscular strength and vigor and great improvement in the patient's sense of mental and physical well being.

Woodyatt in determining the practical limit of the caloric intake, considers the patient's financial status and his ability to pay for large amounts of insulin, the size of the patient and the nature of his life and work. One man may be able to attend to his daily duties with 2000 calories while a larger man doing heavier work may require more. From the economic viewpoint a certain individual may be able to do more efficient work with larger doses, thereby increasing his earning capacity.

Allen and Sherrill call attention to the increased danger of death from coma in patients receiving a high caloric and high fat intake. In children they provide for the demands of growth and development. If a higher diet raises the earning capacity, a higher dosage of insulin may be a good investment even for a day laborer. Accuracy of control is another factor. Thus a child of wealthy parents who will always be attended by a specially trained, highly skilled nurse, is fed more abundantly than another child treated on a charity basis who will ultimately have to carry the responsibility of his own treatment at home. They emphasize particularly that attention to the diet is more important when insulin is being given than otherwise. "All our attempts with lax or inaccurate diets in conjunction with insulin treatment have led to disastrous results. Insulin has tremendously improved diabetic treatment but has not simplified it."

Effect on Basal Metabolism.—Wilder, Boothby and their coworkers found in certain cases that the basal respiratory quotients and the basal metabolic rates were but little affected by insulin. Fitz, Murphy and Grant, who made a special study of the effect on metabolism, confirmed the conclusions of Banting, Best and their associates in that insulin caused a definite increase in the respiratory quotient. Sometimes the quotients remained up as long as three days after the drug had been discontinued, suggesting that the effect of insulin does not immediately wear off. Joslin, Gray and Root found the basal metabolism increased nine per cent after prolonged treatment in a series of eleven patients. The respiratory quotient rose with food after insulin to above unity.

Effect on Protein and Fat Metabolism.—Banting, Campbell and Fletcher have shown a definite influence of insulin in sparing nitrogen. Fitz, Murphy and Grant found an apparent influence on protein katabolism, enabling the diabetic to store nitrogen instead of excreting an amount equal to or greater than that ingested. Insulin evidently spares proteins. Wilder, Boothby and their coworkers found that insulin had less effect with a high protein, low fat diet, than with a relatively higher fat and lower protein diet, the caloric

content and carbohydrate content of each diet being the same. Allen and Sherrill on the other hand, report that these differences are greatest in short tests and in the long run are not sufficient to warrant the incovenience and discomfort, if not actual danger, of an overbalanced ratio. They follow no fixed rules of protein requirement or ketogenic-antiketogenic balance, but content themselves with giving a safe sufficiency of protein and carbohydrate. They commonly allow in a diet of 2000 calories, 100 grams protein and 100 to 150 grams carbohydrate. The difference in the insulin requirement as compared with a diet composed more largely of fat is relatively slight in the long run, while there are important differences in comfort, in the ability to eat articles customarily found in the normal diet, and in freedom from acidosis dangers.

Must the Patient Be Maintained "Sugar Free"?—There appears some diversity of opinion among the various observers as to whether for best results the patient's diet and insulin dosage should be so regulated that he remains entirely free from glycosuria. There is also divergence of opinion as to how near to normal the blood sugar should be maintained. Banting, Campbell and Fletcher remark that blood sugars well above normal provide some protection against the occurrence of hypoglycemia and also allow greater economy in the use of insulin, but that it hardly seems justifiable to abandon as a principle in treatment the maintenance of the blood sugar at a normal level. Hyperglycemia may be a factor in some of the degenerative processes such as arteriosclerosis, optic neuritis and so on, and besides blood sugar above the normal level must throw a greater load upon the islet tissue of the pancreas. They attempt to keep the patient sugar free.

Joslin, Gray and Root brought an average fasting blood sugar of 0.24 per cent down to 0.19 per cent at the end of treatment. They also attempt to keep the patient sugar free. Geyelin has purposely never totally abolished glycosuria for any successive period longer than ten days. He and his associates allow a moderate glycosuria in all cases as the most practical method of lessening the possibility of insulin overdosage. They attempt to maintain the glycosuria at ten grams or less per day. Woodyatt finds that owing to unavoidable variations in diet, insulin dosage, exercise, etc., the old cases with low fixed tolerance limits are kept sugar free with difficulty, and he believes it unnecessary to keep this type sugar free because they have little or no natural tolerance to lose. On the other hand, all those having a considerable natural tolerance or power to regain such should be kept sugar free if possible.

Allen and Sherrill favor maintaining the urine free from sugar. They point out that the loading of urine and blood with sugar is decidedly abnormal and that the remote effects thereof will be bad. If thorough treatment is preferable in the emergency conditions it is difficult to see why it should not be equally advantageous before their development. They call attention to the lowered resistance to infection, arteriosclerosis, gangrene, etc., which may follow hyperglycemia, and insist that lax treatment with insulin does no more than convert the case into a milder but still active form of diabetes.

They find it feasible for patients who have received proper instruction to remain free from glycosuria and nearly free from hyperglycemia on their return home.

Does Insulin Produce a Permanent Increase of Tolerance?—Uppermost in the minds of all since the advent of insulin has been the question—"will its administration produce permanent cure?" This has been the hope of all sufferers from the disease. Banting, Campbell and Fletcher feel at present when no case has been under continuous treatment for as long as a year, that it is too early to prognosticate remote results. They find that such evidence as we have indicates that the disease may be satisfactorily controlled even after the patients have left the hospital. They do not claim definite permanent improvement in sugar tolerance. Some cases have, however, given rather suggestive results. They have estimated the carbohydrate utilization power after insulin in cases who had previously been under prolonged dietary treatment. They find that certain patients do regain some increased power of carbohydrate utilization under insulin therapy. Increases in total glucose tolerance of as much as twenty to thirty grams have several times been observed. This increase persists as long as excessive demands are not made upon the pancreas by excessively high diet or high blood sugar levels. In other patients no increase in carbohydrate tolerance has been obtained.

Joslin, Gray and Root do not accept entirely the conclusion of Allen and Sherrill that diabetes is a nonprogressive disease. They are not sure that even under insulin therapy it is not a progressive disease. Thus Miss N. after several months' treatment, has rather more difficulty in keeping free from glycosuria with 20 units daily than she had formerly. They stress that at the beginning of treatment and until a balance is obtained, more insulin will be required, but that the subsequent decrease to a smaller amount does not indicate an increased tolerance. This latter level of insulin dosage cannot be further decreased.

They emphasize that the diabetic on dietary treatment alone, the patient with subnormal metabolism, has a different type of disease and cannot be compared in all features with a diabetic receiving insulin, whose metabolism is nearly normal. They caution against the production of the diabetic with increased metabolism. Slight increases in insulin requirement early in treatment may be explained by the resulting increase in metabolism rather than diminution on the power of insulin to act.

Williams in a group of severe cases found it necessary to practically double the amount of extract required when the treatment was first instituted. In each case, however, there resulted a gain in weight of from 15 to 25 pounds and an even greater gain in strength. In three cases there appeared to be considerable gain in ability to utilize glucose. In several other cases treated for shorter periods of time and notably in children, it was found necessary to gradually increase the dose from 20 to 50 per cent. This suggests a gradual failure in pancreatic function or response to the drug. None of his 43 cases showed evidence of increased pancreatic func-

tion. A gradual decline in function indicates the necessity for continuance of careful dieting.

Allen and Sherrill point out that gain in weight naturally calls for an increase in insulin dosage. Three diabetics seemed to lose tolerance demonstrably when glycosuria was permitted along with insulin treatment. Several others have shown gain of assimilative power with insulin beyond anything that could be expected from dietary treatment alone.

Renal Diabetes.—Doses of five and ten units of insulin have exerted little effect on the glycosuria in several cases examined by Woodyatt. He does not state definitely regarding the blood sugar.

Conclusions.—The researches so far reported indicate that insulin supplies in its entirety whatever metabolic deficiency the diabetic patient possesses. This is an important point in evidence for those who contend that diabetes is primarily and entirely due to disturbed pancreatic function. Its use revolutionizes the treatment of diabetes but it does not simplify it. Its abuse may jeopardize the patient. It should not be used to fatten individuals unnecessarily nor to enable an obese diabetic to remain obese. The drug is powerful both in its beneficial and in its harmful effects and should only be used with a full understanding of both.

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—*W. T. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

*Standard Methods of Milk Analysis**

THE American Public Health Association and the Association of Official Agricultural Chemists established in 1922 a series of standard methods of milk analysis, including bacteriological and chemical examination and methods for the detection of preservatives, coloring matters, etc. These standard methods were published in a pamphlet of forty pages, which is now in its fourth edition.

*Standard Methods of Milk Analysis. Of the American Public Health Association and the Association of Official Agricultural Chemists. Fourth edition. Paper. Price forty cents. Pp. 40. American Public Health Association, New York, 1923.

*Practical Physics**

THE feature which immediately appeals in this book is the successful attempt made by the author to so arrange physical laboratory experiments that a minimum of special apparatus is necessary. In fact a great proportion of the experiments may be carried out with no other equipment than such articles as those with which we come in daily contact. Such for instance is an experiment to determine the area of an irregular figure by means of squared paper, or an experiment to determine the circumference of a cylinder by multiple windings of an ordinary thread. The work fulfills the title of the book in that it is practical in every sense and at all times keeps before the student a realization that physics is primarily not a matter of expensive laboratory equipment, but is a phase of the every day problems of life.

The volume is a laboratory manual rather than a text book and is so arranged as to promote interest in the work and individuality in solving problems.

ThePhysiology of Twinning†

THE PHYSIOLOGY OF TWINNING, dealing primarily with the causes and consequences of twinning, is an elaboration of an earlier volume entitled The Biology of Twins, published in 1917. The book serves as a vehicle for the exposition of the author's theories regarding the production of twins and monsters in general. Much of the discussion is devoted to consideration of the comparative physiology or pathology, as one may choose to call it, of twinning. In it is an able résumé of the author's studies and of the studies of others on the phenomenon in the starfish, the earthworm, in fishes, birds, amphibiæ, reptiles, armadillos, and in mammalia particularly human beings.

Early in the book the author summarizes his theory which is briefly that twinning is a phenomenon of the physiologic isolation of equivalent parts of the blastoderm and regulation of the isolated or twinned regions into complete embryos. The cause of the physiologic isolation may be a temporary cessation or radical retardation of development at a critical period, such that the polarity of the embryo becomes weakened and there are no longer high and low metabolic regions. All parts of the embryo are left on a parity. When normal developmental conditions return, some new part of the embryo becomes the apical point for the establishment for a new or additional gradient. There thus develop two or more growing points or axes of growth. The application of existing facts to this theory is developed throughout the remainder of the volume. The work and theories of others are given due recognition.

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*Practical Physics. By J. A. Crowther, Sc.D., F. Inst. P., sometime Fellow of St. John's College, Cambridge; Demonstrator of Physics in the Cavendish Laboratory; University Lecturer in Physics as applied to Medical Radiology, Cambridge. Cloth. Price, $3.25. Pp. 260. Henry Frowde and Hodder & Stroughton. London. 1922.

†The Physiology of Twinning. By Horatio Hackett Newman, Professor of Zoology, University of Chicago. Cloth. Pp. 230. The University of Chicago Press, Illinois, Price, $1.85. 1923.

*Ortner on Pain**

A RATHER extensive treatise by this distinguished Viennese physician has recently appeared in an English translation. It consists of two volumes, the first devoted to a discussion of abdominal pain, the second, although entitled Generalized Pain, dealing chiefly with regional pain originating in portions of the body other than the abdomen. Many volumes have been written on the single symptom pain and it has been dealt with by as many methods. From the breadth of his own clinical experience, Ortner is able to enumerate practically all of the known causes of pain referable to each region of the body. This he does in a didactic manner. Having enumerated the possible causes, he gives scant attention, as a rule, to true differential diagnosis.

The two volumes have not been translated by the same individual. That on abdominal pain is easy reading and an excellent translation. That on generalized pain, whatever its intrinsic value in the original, has lost much thereof in the translation.

The Chemistry of Tuberculosis†

THE collaborators responsible for this volume have devoted many years to study of the chemistry of the tubercle bacillus and its environment. By environment, we refer not only to changes in artificial culture media which influence or are influenced by the growth of the tubercle bacillus, but also to reactionary changes in the living host. They have had occasion to review in this connection a tremendous mass of literature. From this they have sorted the work of undoubted value, and the present volume stands as an unusually complete compilation thereof. The subject matter treated would at first appear to interest but a few individuals, chiefly research students in the field of tuberculosis. Nevertheless the work is excellently done and at many points touches on practical features in the study of clinical tuberculosis. It will without doubt be of practical value to all interested in tuberculosis, whether from a clinical or a research point of view. The literature on each phase of the chemistry of tuberculosis is reviewed impersonally. At the close of each chapter, there appears a recapitulation in which the authors have permitted themselves some critical discussion, which enhances the value of the treatise. The chapter on Specific Chemotherapy is of particular interest and the conclusions are thoroughly conservative.

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*Abdominal Pain. By Prof. Dr. Norbert Ortner, Chief of the Second Medical Clinic at the University of Vienna. Authorized Translation by William A. Brams, M.D. Formerly Lieutenant-Commander, Medical Corps, U.S.N. and Dr. Alfred P. Luger, First Assistant, Second Medical Clinic, University of Vienna. Cloth. Price $4.00. Pp. 362. Rebman Company, New York, 1922.

Generalized Pain. By Prof. Dr. Norbert Ortner, Vienna. Only authorized Translation into the English Language of the 2nd German edition by Francis J. Rebman. With an introduction by Thomas Webster Edgar, M.D. Cloth. Price, $4.00. Pp. 596. Medical Art Agency, New York, 1922.

†The Chemistry of Tuberculosis. Being a compilation and critical review of existing knowledge on the chemistry of the tubercle bacillus and its products, the chemical changes and processes in the host, the chemical aspects of the treatment of tuberculosis. By H. Gideon Wells, M.D., Ph.D. Director of the Otho S. A. Sprague Memorial Institute, Professor of Pathology in the University of Chicago and in Rush Medical College. Lydia M. DeWitt, M.D., A.M. Member of the Otho S. A. Sprague Memorial Institute, Associate Professor of Pathology in the University of Chicago and in Rush Medical College. Esmond R. Long, Ph.D. Assistant Professor of Pathology in the University of Chicago and in Rush Medical College. Cloth. Price, $5.00. Pp. 447. Williams and Wilkins Company, Baltimore, 1923.

*Colloids in Biology and Medicine**

CHEMISTRY in general and physiological chemistry or biochemistry in particular, aims to investigate the structure of individual chemical substances and to explain their properties by splitting and synthesizing them and comparing the rearticulated substances with the original. Colloidal substances, although susceptible to analysis, cannot be entirely resynthesized, and the methods of colloidal research are therefore quite different from those customarily used in organic and physiologic chemistry. Colloidal chemistry consists primarily in the study of the machine as a whole rather than of its component parts. The splitting of proteins into peptones and amino acids is a violent procedure and the study of the resultant split products gives us little thorough information of the nature of the mother substance. The student of biocolloids avoids such profound attacks and strives to keep the molecule intact so far as possible, studying its outward form, the chemical points of attack offered by the unmutilated molecule and its behavior to changes which may occur under normal and pathological conditions.

Colloid chemistry thus becomes a division of chemistry quite apart from the other more familiar methods of study. It approaches more nearly a study of the living molecule.

After a detailed consideration of colloids, colloidal phenomena and methods of colloidal research, the author enters into a discussion of various colloids as individuals, particularly lipoids, proteins and carbohydrates. The major portion of the book is devoted to a practical discussion of colloidal phenomena as they occur in the living body. These studies are grouped under the headings Enzyme Action, Immunity Reactions, Metabolism of the Various Tissues and Systems of the Body, Growth, Metamorphosis and Development, Secretion and Excretion. The chapters on the movements of organisms, blood respiration and circulation are of particular interest. The colloidal properties of foods and condiments and colloidal phenomena as they occur in toxicology and pharmacology are discussed.

As usual, when one becomes intensely interested in a single subject, the author observes the majority of phenomena in terms of colloidal chemistry.

On the whole, the volume may be characterized as reasonably conservative, thoroughly comprehensive, and as one, a study of which offers the solution to many problems in medicine which have not been satisfactorily answered in the phraseology of physiological chemistry.

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*Colloids in Biology and Medicine. By Prof. H. Bechhold, Member of the Royal Institute for Exp. Therapeutics in Frankfort A. M. Authorized Translation from the second German edition with notes and emendations by Jesse G. M. Bullowa, A.B.M.D., Assistant Clinical Professor of Medicine, Fordham University; Adjunct Professor of Clinical Medicine, N. Y. Polyclinic School and Hospital; Visiting Physician Riverside Sanatorium; Associate Visiting Physician Willard Parker Hospital, N. Y. City. Price $5.00. Pp. 464. 54 illustrations. D. Van Nostrand Co., New York, 1919.